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## **(54) HEMATOPOIETIC ORGAN TUMOR CELL DETECTION METHOD AND HEMATOPOIETIC ORGAN TUMOR CELL DETECTION KIT**

(57)Abstract:

**PROBLEM TO BE SOLVED:** To provide a hematopoietic organ tumor cell detection method which detects with high sensitivity and high specificity a hematopoietic organ tumor cell, and a detection kit.

**SOLUTION:** The hematopoietic organ tumor cell detection method comprises the steps of: quantifying a tyrosine phosphatase SHP1 protein specific to a hematopoietic organ cell included in a specimen sample containing the hematopoietic organ cell; and identifying methylation of CpG islands included in the base sequencing of the SHP1 gene obtained from the above-mentioned specimen sample. Thus, since the presence or absence of the hematopoietic organ tumor cell is identified at two stages by one genetic information, the hematopoietic organ tumor cell is detectable by a very high specificity.

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## CLAIMS

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[Claim(s)]

[Claim 1]

(1) A SHP1 gene-methylation check process which checks methylation of a CpG island included in a base sequence of protein tyrosine phosphatase SHP1 gene specific into a hematopoietic organ cell included in a sample sample containing a hematopoietic organ cell,

(2) a SHP1 gene-product fixed-quantity process of quantifying at least one expression amount of SHP1 protein obtained from the above-mentioned sample sample, and SHP1mRNA -- and

(3) A SHP1 gene LOH check process which checks existence of heterozygosity loss (LOH) of SHP1 gene contained in the above-mentioned sample sample,

\*\* -- a hematopoietic organ tumor cell detection method by which it being included any they are even if small.

[Claim 2]

In the above-mentioned SHP1 gene-methylation check process

A gene cutting trial stage processed with a methylation sensitivity restriction enzyme which recognizes a base sequence which includes a genetic material obtained from the above-mentioned sample sample for cytosine,

A gene amplification trial stage of enforcing a polymerase chain reaction method (PCR) using a primer which amplifies a field which is included in a base sequence of the SHP1 above-mentioned gene, and includes a base sequence by which recognition cutting is carried out in the above-mentioned methylation sensitivity restriction enzyme to a gene processed with the above-mentioned methylation sensitivity restriction enzyme,

The hematopoietic organ tumor cell detection method according to claim 1, wherein the amount check stage of gene amplification of checking quantity of a gene of amplified specific size is included.

[Claim 3]

The hematopoietic organ tumor cell detection method according to claim 2, wherein the above-mentioned primer is a partial base sequence further included in a base sequence shown in the array number 1 or 2, or the polynucleotide which has this partial base sequence and complementarity.

[Claim 4]

The hematopoietic organ tumor cell detection method according to claim 2 or 3 characterized by checking quantity of a gene of specific size using an electrophoresis method in the above-mentioned amount check stage of gene amplification.

[Claim 5]

The hematopoietic organ tumor cell detection method according to claim 2, 3, or 4 characterized by using a methylation sensitivity restriction enzyme as a restriction enzyme in the above-mentioned gene cutting trial stage.

[Claim 6]

In the above-mentioned SHP1 gene-methylation check process

A gene ornamentation stage of processing a genetic material obtained from the above-mentioned sample sample by a bisulfite,

The hematopoietic organ tumor cell detection method according to claim 1, wherein a methylation cytosine content judging stage included in a genetic material processed by a bisulfite

of judging existence of methylation cytosine in a base sequence of SHP1 gene is included.

[Claim 7]

A way PCR detects methylation cytosine in the above-mentioned methylation cytosine content judging stage, The hematopoietic organ tumor cell detection method according to claim 6, wherein it is used at least among a way determination of a base sequence of a gene detects methylation cytosine, or a method of identifying a base sequence containing methylation cytosine any they are.

[Claim 8]

The hematopoietic organ tumor cell detection method according to claim 6 or 7 characterized by using sodium bisulfite as a bisulfite in the above-mentioned gene ornamentation stage.

[Claim 9]

A hematopoietic organ tumor cell detection method given in any 1 paragraph of claims 1 thru/or 8 quantifying SHP1 protein in the above-mentioned SHP1 gene-product fixed-quantity process using SHP1 antibody which uses SHP1 protein as an antigen.

[Claim 10]

The hematopoietic organ tumor cell detection method according to claim 9 characterized by quantifying SHP1 protein by enzyme-labeled antibody technique or a western blotting method in the above-mentioned SHP1 gene-product fixed-quantity process.

[Claim 11]

By detecting a manifestation of mRNA of SHP1 gene using polynucleotide which detects an overall length of a base sequence, or its part of SHP1 gene cDNA shown in the array number 3 in the above-mentioned SHP1 gene-product fixed-quantity process, A hematopoietic organ tumor cell detection method given in any 1 paragraph of claims 1 thru/or 8 quantifying SHP1 mRNA.

[Claim 12]

In the above-mentioned SHP1 gene-product fixed-quantity process, a northern blotting method, The reverse transcription PCR method, the real-time PCR method, or RNA in The hematopoietic organ tumor cell detection method according to claim 11, wherein a manifestation of mRNA of SHP1 gene is detected by a situ hybridization method.

[Claim 13]

Two Microsatellite markers which put the SHP1 above-mentioned gene at least a check of existence of heterozygosity loss on the other hand, Or a hematopoietic organ tumor cell detection method given in any 1 paragraph of claims 1 thru/or 12 carrying out gene polymorphism like simple nucleotide polymorphism which exists the inside of the above-mentioned SHP gene, and near it in the fragmentation analysis using PCR.

[Claim 14]

It is used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell,

(1) SHP1 antibody which uses protein tyrosine phosphatase SHP1 protein specific into a hematopoietic organ cell as an antigen -- and

(2) A methylation sensitivity restriction enzyme which recognizes a base sequence containing cytosine,

A hematopoietic organ tumor cell detection kit comprising:

A primer for PCR which amplifies a field which is included in a base sequence of SHP1 gene and includes a base sequence recognized by the above-mentioned methylation sensitivity restriction enzyme.

the methylation positivity of the SHP1 above-mentioned gene, and inside with methylation

negative control DNA -- at least -- on the other hand.

[Claim 15]

It is used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell,

(1) SHP1 antibody which uses protein tyrosine phosphatase SHP1 protein specific into a hematopoietic organ cell as an antigen,

(2) a primer for a judgment of existence of cytosine in a base sequence of SHP1 gene contained in a genetic material processed by bisulfite refined to a gene processing level, and this bisulfite -- and

(3) A hematopoietic organ tumor cell detection kit containing any at least one of primers for PCR which detect an overall length of a base sequence, or its part of SHP1 gene cDNA shown in the array number 3.

[Claim 16]

It is used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell,

A hematopoietic organ tumor cell detection kit containing a primer for PCR which detects at least one overall length of two Microsatellite markers which put protein tyrosine phosphatase SHP1 specific gene between a hematopoietic organ cell, or its part.

[Claim 17]

The hematopoietic organ tumor cell detection kit according to claim 14, 15, or 16 containing either [ at least ] a reagent for PCR, or a reagent for a restriction enzyme reaction.

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## DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention]

This invention relates to the hematopoietic organ tumor cell detection kit used suitably for a hematopoietic organ tumor cell detection method and this detecting method.

By detecting methylation of SHP1 gene which encodes manifestation reduction, disappearance, or this of protein tyrosine phosphatase SHP1 specific gene product to a malignant lymphoma, leukemia, etc. especially, for example, It is related with the detecting method and detection kit which can detect hematopoietic organ tumor cells on high sensitivity and a high unique target.

[0002]

[Description of the Prior Art]

Various kinds from what has a very bad prognosis to what has a comparatively good prognosis are known for intractableness by hematopoietic organ tumors (tumor of blood systems) in Homo sapiens (Homo sapiens), such as a malignant lymphoma and leukemia. Although various therapies, such as various chemotherapies, radiotherapy, or immunotherapy, are already put in practical use by the therapy of this hematopoietic organ tumor, even if tumor cells carry out regression mostly as a result of such a therapy, if tumor cells survive slightly, the relapse of a hematopoietic organ tumor will not be escaped.

[0003]



Diagnosis of the above-mentioned hematopoietic organ tumor is synthetically carried out by using two or more modalities together by the former. Using peripheral blood and various biopsy specimens, the morphological observation and the histological observation by a tissue staining color, immunity dyeing, etc. are carried out, or, specifically, further various molecular biological analyses, chromosome analyses, etc. are carried out. Diagnosis of the above-mentioned hematopoietic organ tumor takes time most by judgment.

[0004]

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## TECHNICAL PROBLEM

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[Problem(s) to be Solved by the Invention]

each above-mentioned conventional modality -- it -- if independent -- hematopoietic organ tumor cells -- high sensitivity -- high -- it is not specifically and promptly detectable. So, in the modality of these former, if plurality is used together and it does not judge synthetically, a hematopoietic organ tumor cannot be diagnosed.

[0005]

That is, as long as the conventional modality is used, in order to have to use two or more modalities together, it not only causes complicated-ization of diagnosis, but time is taken, and since neither hematopoietic organ tumor cell detection sensitivity nor singularity is high, a medical practitioner's special judgment will occupy big specific gravity to diagnosis. Therefore, in the former, the diagnostic technique of a hematopoietic organ tumor is not used for the purpose of the early detection and early treatment of the hematopoietic organ tumor by a mass screening, although it is substantially restricted to use in a medical site and can respond to each disease person.

[0006]

In order to carry out hematopoietic organ tumor cells on high sensitivity and a high unique target more, it is possible to use the marker with high sensitivity specifically looked at by the hematopoietic organ tumor of the wide range to hematopoietic organ tumor cells. If such a marker is used, early detection and diagnosis of a hematopoietic organ tumor can be carried out easily and promptly, It not only becomes possible to apply to the early treatment and recurrence prevention of a malignant lymphoma, leukemia, etc., but on medical science, it can consider it as available diagnostic technique at clinical laboratory test industry, pharmaceutical industry, etc., and it becomes possible to contribute to development of industry. However, such a marker is not known the place by the present.

[0007]

This invention is made in view of an aforementioned problem, and the purpose, Using molecular biological knowledge, hematopoietic organ tumor cells are detected from a small amount of patient samples on high sensitivity and a high unique target promptly and simple, the early detection, diagnosis, and early treatment of a hematopoietic organ tumor are made easy, and it is in providing a hematopoietic organ tumor cell detection method and a detection kit applicable also to a mass screening.

[0008]

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## MEANS

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[Means for Solving the Problem]

As a result of inquiring wholeheartedly in view of an aforementioned problem, this invention persons in a large malignant hematopoietic organ tumor of a range. In a hematopoietic organ tumor with malignancy high moreover in which expression inhibition of protein tyrosine phosphatase SHP1 protein is extremely seen by high frequency, By using as a marker both sides of SHP1 gene which finds out that a tendency of expression inhibition of the SHP1 above-mentioned protein becomes strong, and encodes SHP1 gene product and this. high sensitivity of hematopoietic organ tumor cells -- high -- a thing [ specific and ] for which hematopoietic organ tumor cell detection art which can be detected in a short time and can be used industrially can be realized is found out, and it came to complete this invention.

[0009]

Namely, a hematopoietic organ tumor cell detection method concerning this invention, . In order to solve the above-mentioned technical problem, are contained in a sample sample containing (1) hematopoietic organ cell. A SHP1 gene-product fixed-quantity process of quantifying at least one expression amount of protein tyrosine phosphatase SHP1 specific protein and SHP1mRNA into a hematopoietic organ cell, (2) A SHP1 gene-methylation check process which checks methylation of a CpG island included in a base sequence of SHP1 gene acquired from the above-mentioned sample sample, And it is characterized by including at least one side of SHP1 gene LOH check process \*\* which checks existence of heterozygosity loss (LOH) of SHP1 gene contained in the (3) above-mentioned sample sample.

[0010]

This phenomenon is not looked at by normal blood cell to expression inhibition of the SHP1 above-mentioned gene product being extremely looked at by malignant hematopoietic organ tumor cells by high frequency. Expression inhibition of the SHP1 above-mentioned protein is based on methylation of SHP1 gene. One allele of SHP1 gene has lost before and after transfer control of SHP1 gene by DNA methylation.

[0011]

By according to the described method, checking methylation of SHP1 gene obtained from a sample sample using the above-mentioned knowledge, and detecting existence of hematopoietic organ tumor cells. screening existence of existence of malignant hematopoietic organ tumor cells -- on the other hand -- SHP in a sample sample -- a manifestation of SHP1 protein, SHP1mRNA, or its both is specifically quantified 1 gene product.

[0012]

That is, in a described method, inactivation of SHP1 gene can be judged using four-fold marker by the maximum called loss of ornamentation and mRNA of gene DNA, protein, and allele. namely, one hematopoietic organ tumor cell called a SHP1 gene-expression fall -- since a specific phenomenon can be checked in four steps, hematopoietic organ tumor cells are detectable by very high singularity.

[0013]

As a desirable example of a hematopoietic organ tumor cell detection method concerning this invention, A gene cutting trial stage processed with a methylation sensitivity restriction enzyme which recognizes a base sequence which includes in the above-mentioned SHP1 gene-methylation check process a genetic material obtained from the above-mentioned sample sample for cytosine, It is contained in a base sequence of the SHP1 above-mentioned gene to a gene processed with the above-mentioned methylation sensitivity restriction enzyme, A detecting method with which a gene amplification trial stage of enforcing the PCR method using a primer

which amplifies a field including a base sequence by which recognition cutting is carried out, and the amount check stage of gene amplification of checking quantity of a gene of amplified specific size are included in the above-mentioned methylation sensitivity restriction enzyme can be mentioned.

[0014]

According to the described method, after distinguishing existence of methylation by trying cutting of SHP1 gene contained in a genetic material obtained from a sample sample using a methylation sensitivity restriction enzyme and amplifying using PCR further, quantity of an PCR product of specific size obtained is checked. So, if even a little SHP1 genes are obtained from a sample sample, methylation of SHP1 gene is detectable. Therefore, even if hematopoietic organ tumor cells exist very much in a sample sample only in a minute amount, it is high detection sensitivity, and it becomes possible to detect hematopoietic organ tumor cells on a high unique target moreover.

[0015]

In the above-mentioned detecting method, it is preferred that the above-mentioned primer is a partial base sequence further included in a base sequence shown in the array number 1 or 2 or the polynucleotide which has this partial base sequence and complementarity.

[0016]

In the above-mentioned detecting method, it is preferred to check quantity of a gene of specific size in the above-mentioned amount check stage of gene amplification using an electrophoresis method.

[0017]

In the above-mentioned detecting method, it is preferred to use a restriction enzyme in which a methylation insensitive restriction enzyme which recognizes the same base sequence is known as a methylation sensitivity restriction enzyme in the above-mentioned gene cutting trial stage.

[0018]

As other examples with a preferred hematopoietic organ tumor cell detection method concerning this invention, A gene ornamentation stage of processing a genetic material obtained from the above-mentioned sample sample by a bisulfite to the above-mentioned SHP1 gene-methylation check process, A detecting method with which a methylation cytosine content judging stage included in a genetic material processed by a bisulfite of judging existence of methylation cytosine in a base sequence of SHP1 gene is included can be mentioned.

[0019]

If a genetic material obtained from a sample sample using a bisulfite is processed according to the described method, cytosine in a base sequence will be changed into uracil, but methylated cytosine is not changed. Therefore, methylation of SHP1 gene is detectable only by judging whether cytosine is contained in a base sequence of SHP1 gene after a gene ornamentation stage. Therefore, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by a simple mechanism.

[0020]

In the above-mentioned detecting method, in the above-mentioned methylation cytosine content judging stage. It is desirable even if it is used at least among processings of a gene by way PCR detects methylation cytosine, way determination of a base sequence of a gene detects methylation cytosine, or a method of identifying a base sequence containing methylation cytosine any they are.

[0021]

By using PCR at least according to the described method, if even a little SHP1 genes are obtained from a sample sample, methylation of SHP1 gene is detectable. Therefore, even if hematopoietic organ tumor cells exist very much in a sample sample only in a minute amount, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by high detection sensitivity.

[0022]

In the above-mentioned detecting method, it is preferred that sodium bisulfite is used as a bisulfite in the above-mentioned gene ornamentation stage. In the above-mentioned gene ornamentation stage, urea may be used together with a bisulfite.

[0023]

In a hematopoietic organ tumor cell detection method concerning this invention, even if it is a detecting method of which example of the above, when SHP1 protein is quantified in the above-mentioned SHP1 gene-product fixed-quantity process using SHP1 antibody which uses SHP1 protein as an antigen, it is desirable. Specifically in the above-mentioned SHP1 gene-product fixed-quantity process. It is desirable when SHP1 protein is quantified by enzyme-labeled antibody technique (an immunohistochemical method, an immunocytochemistry method, the ELISA (enzyme-linked immunosorbent assay) method) or a western blotting method.

[0024]

Since SHP1 protein will be quantified using an antigen-antibody reaction according to the described method, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by a simple mechanism.

[0025]

Or in a hematopoietic organ tumor cell detection method concerning this invention, Even if it is a detecting method of which example of the above, in the above-mentioned SHP1 gene-product fixed-quantity process. By detecting a manifestation of mRNA of SHP1 gene using polynucleotide which detects an overall length of a base sequence, or its part of SHP1 gene cDNA shown in the array number 3, even if it quantifies SHP1 mRNA, it is desirable. Specifically at the above-mentioned SHP1 gene-product fixed-quantity process, they are a northern blotting method, the reverse transcription PCR method, the real-time reverse transcription PCR method, or RNA in. It is desirable when a manifestation of mRNA of SHP1 gene is detected by a situ hybridization method.

[0026]

Since SHP1 gene product will be quantified by mRNA of SHP1 gene as SHP1 gene product according to the described method, a mechanism simple by using oligopeptide which has cDNA and homology of SHP1 gene as a probe or a primer -- high -- it becomes possible specific and to detect hematopoietic organ tumor cells to high sensitivity.

[0027]

a hematopoietic organ tumor cell detection method concerning this invention -- it is desirable -- as other examples to a pan, Two Microsatellite markers which put the SHP1 above-mentioned gene at least a check of existence of heterozygosity loss on the other hand, Or a method enforced by [ which used PCR ] conducting fragmentation analysis in gene polymorphism like simple nucleotide polymorphism which exists the inside of the above-mentioned SHP1 gene and near it can be mentioned. The sample sample used at this time should just be a sample sample containing a hematopoietic organ cell. As contrast, a sample obtained after complete hematological remission may be used, and other normal tissue cells may be used.

[0028]

Since heterozygosity loss of SHP1 gene is checked by checking heterozygosity loss of gene polymorphism, such as a Microsatellite marker or simple nucleotide polymorphism (SNP), by PCR according to an above-mentioned method, It becomes possible to detect hematopoietic organ tumor cells more certainly by a simple mechanism.

[0029]

As a desirable example of a hematopoietic organ tumor cell detection kit concerning this invention, It is used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell, (1) A methylation sensitivity restriction enzyme which recognizes a base sequence which contains in a hematopoietic organ cell SHP1 antibody which uses protein tyrosine phosphatase SHP1 specific protein as an antigen, and (2) cytosine, Composition containing at least one side can be mentioned among a primer for PCR which amplifies a field which is included in a base sequence of SHP1 gene and includes a base sequence by which recognition cutting is carried out in the above-mentioned methylation sensitivity restriction enzyme, and the methylation positivity of the SHP1 above-mentioned gene and methylation negative control DNA.

[0030]

Or as other examples with a preferred hematopoietic organ tumor cell detection kit concerning this invention, A bisulfite which was used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell, and was refined to (1) above-mentioned SHP1 antibody and (2) gene processing level, A primer for a judgment of existence of cytosine in a base sequence of SHP1 gene contained in a genetic material processed by this bisulfite, And composition containing any at least one of primers with an overall length, or its part and homology of a base sequence of the SHP1 gene cDNA shown in the (3) array number 3 for PCR can be mentioned.

[0031]

As other examples in which a hematopoietic organ tumor cell detection kit concerning this invention is preferred, It is used in order to detect hematopoietic organ tumor cells from a sample sample containing a hematopoietic organ cell, Composition containing a primer for PCR which detects at least one overall length of two Microsatellite markers which put protein tyrosine phosphatase SHP1 specific gene between a hematopoietic organ cell, or its part can be mentioned.

[0032]

In the above-mentioned hematopoietic organ tumor cell detection kit, it is still more preferred that either [ at least ] a reagent for PCR or a reagent for a restriction enzyme reaction is included.

[0033]

Even if it is which composition of the above, in order to enforce a hematopoietic organ tumor cell detection method mentioned above, desirable drugs, a specimen, etc. are contained. Therefore, a hematopoietic organ tumor cell detection method concerning this invention can be enforced easily and promptly by using the above-mentioned detection kit, and it becomes possible to use this invention on industrial levels, such as clinical laboratory test industry and pharmaceutical industry.

[0034]

[Embodiment of the Invention]

[Embodiment 1]

It will be as follows if one gestalt of the operation in this invention is explained based on drawing 1 thru/or drawing 24. This invention is not limited to this.

[0035]

While this invention quantifies protein tyrosine phosphatase SHP1 gene product, i.e., SHP1 protein, and mRNA from a promotor specific into a hematopoietic organ cell which are contained in the sample sample containing a hematopoietic organ cell, It is the art of detecting hematopoietic organ tumor cells out of the above-mentioned sample sample by checking the methylation of the CpG island included in the base sequence of SHP1 gene acquired from the above-mentioned sample sample.

[0036]

SHP1 gene used as a marker for detecting hematopoietic organ tumor cells by this invention, Exist in the chromosome 12p13 and the base sequence shown in drawing 1 - drawing 10, and the array number 1 is made into the sense strand of genomic DNA (wild type), It is a gene which has an exon (a figure and an array table Chuo University field shown in written form) of 16 which makes an antisense strand the base sequence shown in drawing 11 - drawing 20, and the array number 2. The cDNA has the size of about 1.8 kbs which have a base sequence shown in drawing 21 and the array number 3. SHP1 gene is the same gene as SH-PTP1, PTP1C, HCP, HCPH, PTPN6, HPTP1C, and SHP-1L.

[0037]

SHP1 protein by which the code is carried out to the SHP1 above-mentioned gene, As it is protein tyrosine phosphatase (PTPase) specific into various hematopoietic organ cells and is shown in drawing 22 by molecular weight 68kD, It has the structure of having two SH2 (Src homology domain 2) fields (270 amino acid residue) which serve as tandem construction at the amino terminal side, a PTPase domain of 246 amino acid residue, and the C terminal side field of 93 amino acid residue. It has an amino acid sequence shown in drawing 23 and the array number 4.

[0038]

In a human hematopoietic organ tumor, for example, a malignant lymphoma, and leukemia. The strong expression inhibition of SHP1 protein is seen by not less than 90% of high frequency by many kinds (for example, refer to American Journal of Pathology, Vol.159, No.4, and October2001:1495-1505 grade). Thus, in malignant hematopoietic organ tumor cells, this phenomenon is not looked at by the normal blood cell to the expression inhibition of the SHP1 above-mentioned protein being extremely seen by high frequency.

[0039]

This invention persons found out uniquely that the expression inhibition of the SHP1 above-mentioned protein made a cause the transfer abnormality by the SHP1 above-mentioned gene being methylated.

[0040]

For example, as shown in drawing 24, in the sense strand (it illustrates up to 181 bases - 2160 bases) of the genomic DNA (wild type) shown in drawing 1 - drawing 10, and the array number 1, promoterregion exists before the exon (figure Chuo University character) of 1001 bases - 1163 bases, but. CG arrangement with which cytosine (C) and guanine (G) are located in a line exists in this neighborhood mostly, and forms the CpG island (CpG island) in it (in drawing 24, shading of a bold letter shows CG arrangement). Although cytosine of this CpG island is not methylated in a normal hematopoietic organ cell, many of cytosine of the above-mentioned CG arrangement is methylated, for example by the malignant cell lymphoma. Of course, methylation of cytosine in this CG arrangement is produced not only like a sense strand but like an antisense strand.

[0041]

Advanced methylation of CpG arrangement in the above-mentioned CpG island prevents transfer of mRNA from DNA of SHP1 gene, and, as a result, production of SHP1 protein is controlled. It is extremely concluded by high frequency by hematopoietic organ tumor cells that this phenomenon was mentioned above. And methylation of DNA in SHP1 gene disappears thoroughly, and very high correlation is seen between the knowledge on molecular biology, and the knowledge on clinical at various hematopoietic organ tumor patients' complete remission term. So, it is guessed that the SHP1 gene-expression control by methylation has played the important role in the onset mechanism of hematopoietic organ tumor cells. So, in this invention, the phenomenon of the above-mentioned SHP1 gene-expression control is used as a marker of hematopoietic organ tumor cells.

[0042]

It also found this invention persons uniquely that one allele of SHP1 gene loses before and after transfer control of SHP1 gene arises according to the DNA methylation mentioned above, when the symptoms of diseases, such as a malignant lymphoma and leukemia, develop. Then, it becomes possible by checking heterozygosity loss of SHP1 gene to check loss of the allele of SHP1 gene. So, heterozygosity loss of SHP1 gene can also be used as a marker of hematopoietic organ tumor cells.

[0043]

In a malignant lymphoma or leukemia, a fall or disappearance of DNA methylation of high frequency, heterozygosity loss of high frequency, and SHP1 gene expression is detected by SHP1 gene, and it is in the tendency for outpatient department SHP1 transgenics to control growth of the cell of a corpuscle system, further at it. Thereby, it is suggested strongly that SHP1 gene is one of the antioncogenes.

[0044]

Then, methylation of the CpG island included according to a SHP1 gene-methylation check process in this invention in the base sequence of SHP1 gene obtained from the above-mentioned sample sample is checked, Either [ at least ] SHP1 protein contained in the sample sample which contains a hematopoietic organ cell at a SHP1 gene-product fixed-quantity process, or mRNA is quantified, and three processes of checking heterozygosity loss of SHP1 gene are further used by a SHP1 gene LOH check process. These processes may be used independently and both sides may be used. In a SHP1 gene-product fixed-quantity process, only SHP1 protein may be quantified, only SHP1 mRNA may be detected and both sides may be detected.

[0045]

By this, it screens by detecting methylation of SHP1 gene in a sample sample first, for example, Then, the detection process of becoming final and conclusive existence of hematopoietic organ tumor cells can be carried out by checking the existence of malignant hematopoietic organ tumor cells by quantifying the manifestation of SHP1 gene product of a sample sample at least by one side of SHP1mRNA and SHP1 protein.

[0046]

Therefore, in this invention, SHP1 gene expression can be judged using four-fold marker by the maximum called loss of ornamentation and mRNA of gene DNA, protein, and allele. namely, one hematopoietic organ tumor cell called a SHP1 gene-expression fall -- since a specific phenomenon can be checked by a three-stage, hematopoietic organ tumor cells are detectable by very high singularity.

[0047]

As mentioned above, it is also checked that it is in the tendency which controls growth of the cell of a corpuscle system by introducing SHP1 gene in this invention. So, SHP1 gene is possible also for using for gene therapy, for example, can expect to control growth of tumor cells by transfecting tumor cells in a SHP1 gene-expression vector.

[0048]

The sample sample used by this invention is not especially limited no matter it may be what sample sample, if it is a sample sample containing hematopoietic organ cells, such as peripheral blood or bone marrow fluid. With the hematopoietic organ cell in this invention, although various blood cells are included, various leucocytes are mentioned especially preferably. More specifically, a lymphocyte (a T cell and a B cell), granulocyte (neutrophil leucocyte, eosinophile leucocyte, basophilic leucocyte), monocyte and a macrophage, a mast cell, a spontaneous killer cell, etc. can be mentioned. Or they may be a hematopoietic stem cell and lymphoid precursors.

[0049]

Therefore, in the sample sample used by this invention. It is good also as a sample sample for analysis which is easy to carry out molecular biology analysis by performing publicly known processing conventionally to the blood and the body fluid which extracted blood, bone marrow fluid or body fluid etc. in which the above-mentioned hematopoietic organ cell is contained from Homo sapiens, could use this as a sample sample as it was, and were extracted.

[0050]

As a hematopoietic organ tumor which can apply this invention, specifically, for example, chronic myelogenous leukemia and a Philadelphia chromosome positive (+ (9; 22) (qq34;q11).)

Various myeloproliferative disorder, such as BCR/ABL chronic myelogenous leukemia, chronic neutrophil leucocyte leukemia, chronic eosinophile leucocyte leukemia / hypereosinophilic syndrome, chronic outbreak nature bone marrow fibrosis, polycythemia vera, essential thrombocytosis, and other myeloproliferative disorder that cannot be classified;

Bone marrow atypicality / myeloproliferative disorder, such as chronic myelogenous monocytic leukemia, atypical chronic-myelogenous-leukemia, and infancy nature myelogenous monocytic leukemia;

Bone marrow atypical syndromes, such as refractory anemia (bone marrow atypical syndrome) accompanied by the intractable hypocytosis (bone marrow atypical syndrome) and superfluous blast cell 5q-syndrome accompanied by the refractory anemia accompanied by ringed sideroblast, refractory anemia without ringed sideroblast, and a multi-series metaplasia, and other bone marrow atypical syndromes which cannot be classified;

The acute myelogenous leukemia (AML) accompanied by a recurrent cytogenetic translocation. for example, AML and AML1(CBF-alpha)/accompanied by + (8; 21) (q22;q22), [ ETO and ] the myelogenous leukemia before acuteness (AML accompanied by + (15; 17) (q22;q11-12) -- and - the --) [ and ] PML/RAR-alpha and unusual marrow eosinophil (inv (16), (p13q22), or + (16; 16) (p13;q11).) AML accompanied by CBF beta/MYH 11X, AML accompanied by 11q23 (MLL) abnormalities, AML accompanied by a multi-series metaplasia with front bone marrow atypical syndrome, AML without a multi-series metaplasia with front bone marrow atypical syndrome, AML related to a therapy, and bone marrow atypical syndrome (related to an alkylating agent --) [ and ] The therapy related to epipodophyllotoxin, or the therapy of other types, AML (a low differentiation type, a thing without maturation, and the thing accompanied by maturation.) which otherwise does not belong to a section acute myelogenous monocytic leukemia, acute monocytic leukemia, acute erythroblast leukemia, acute megakaryocyte leukemia, the acute basophilocytic leukemia, the acute over\*\*\*\*\* vegetation accompanied by



the bone marrow fibrosis, and acute 2 character -- acute-myelogenous-leukemia [, such as sex leukemia ] (AML);

a precursor B cell nature tumor (the precursor B-lymphoblastic leukemia / lymphoma (precursor B cell acute lymphoblastic leukemia).) a mature (tip) B cell nature tumor (B cell chronic lymphocytic leukemia / small lymphocytic lymphoma.) B cell prolymphocytic leukemia, a lymphoplasmacytic lymphoma, spleen verge field B cell lymphoma (+/-villus lymphocyte), Pilliform cell leukemia, plasma cell myeloma (plasmocytoma), the verge type B cell lymphoma outside a MALT type paragraph, \*\*\*\* verge type B cell lymphoma (+/- monocyte type B cell), a follicular lymphoma, a mantle cell lymphoma, diffusion large-sized B cell lymphoma (mediastinum large cell B cell lymphoma, primary exudation lymphoma), and Burkitt B cell nature tumors, such as a lymphoma / Burkitt cell leukemia;

a precursor T cell nature tumor (the precursor T-lymphoblastic leukemia / lymphoma (precursor T cell acute lymphoblastic leukemia).) a mature (tip) T cell nature tumor (T cell prolymphocytic leukemia and T cell granulation lymphocyte leukemia.) Invaded type spontaneous killer cell leukemia, adult T-cell lymphoma and leukemia (HTLV1+), A nasal form paragraph extravasio NK/T cell lymphoma, a \*\*\*\*\* type T cell lymphoma, a \*\*\*\* type gamma-delta T cell lymphoma, A T cell and spontaneous killer cell nature tumors, such as a hypodermic phlegmon Mr. T cell lymphoma, mycosis fungoides / Sezary syndrome, an anaplasia nature large-sized cell lymphoma (T/null cell, a primary skin undifferentiated type), a peripheral T cell lymphoma that does not belong to a section at others, and a blood vessel immunoblast T cell lymphoma; Hodgkin lymphomas, such as a \*\*\*\* lymphocyte superior Hodgkin lymphoma and a classical Hodgkin's lymphoma (a tuberos sclerosis Hodgkin lymphoma (grades 1 and 2), a lymphocyte rich classical Hodgkin's lymphoma, a mixed cellularity Hodgkin's lymphoma, a lymphocyte depletion Hodgkin lymphoma) (Hodgkin's disease);

Although \*\* can be mentioned, it is not limited in particular.

[0051]

The SHP1 gene-product fixed-quantity process in this invention, Especially if it is the method of doing either [ at least / a fixed quantity of ] SHP1 protein in a sample sample, or SHP1mRNA, are not limited, but specifically, The method (mRNA assay) of quantifying SHP1mRNA can be conveniently used by detecting the manifestation of mRNA of the method (protein assay) of quantifying SHP1 protein using SHP1 antibody which uses SHP1 protein as an antigen, and SHP1 gene.

[0052]

First, as the more concrete technique of the above-mentioned protein assay, The western blotting method or enzyme-labeled antibody technique (Immunochemistry) using SHP1 antibody (an immunohistochemical method, an immunocytochemistry method, the ELISA (enzyme-linked immunosorbent assay) method) can be mentioned.

[0053]

SHP1 antibody used with the above-mentioned protein assay, It is not what will be limited especially if it is an antibody which recognizes at least a part of structures of SHP1 protein of having the structure shown in drawing 22, drawing 23, and the array number 4, as an antigenic determinant, and can detect SHP1 protein certainly immunologically, It may be a polyclonal antibody and may be a monoclonal antibody.

[0054]

The SHP1 above-mentioned antibody may be conventionally manufactured by a publicly known method, and SHP1 commercial antibody may be used for it. As a manufacturing method of

SHP1 antibody, if it is a monoclonal antibody, the technique produced by the hybridoma with which it makes it come to unite the mouse spleen lymphocytes which carried out immunity in SHP1 protein, and the marrow cells of a mouse will be mentioned, for example. If the SHP1 above-mentioned antibody is a polyclonal antibody, the technique refined from the immune serum of the rabbit which carried out immunity in SHP1 protein will be mentioned. #SH-PTP1(D-11):sc7289 and #SH-PTP1(C-19):sc287 (product made from Santa Cruz Biotechnology Inc.), as SHP1 commercial antibody, # anti SHPTP (06117) and #anti mouse SHPTP (05281) (Product made from Upstate Biotechnology Inc.) etc. -- it is mentioned.

[0055]

The enzyme-labeled antibody technique (an immunohistochemical method, an immunocytochemistry method, the ELISA method) using the SHP1 above-mentioned antibody, A conventionally publicly known method (for example, it, and) [ Kazuo-Nakane/ "enzyme-labeled-antibody-technique"-Keiichi-Watanabe and /-] Interdisciplinarity plan publication (Showa 61) and Brown R.W. et al: Modern Pathol.199;8 (5) The method currently indicated by articles, such as 515-20 (1995), can be used conveniently, Neither in particular the concrete process, nor reagents, conditions, etc. are limited.

[0056]

Similarly the western blotting method using the SHP1 above-mentioned antibody, a conventionally publicly known method (for example, editing besides "experiment operation blotting method" Yoshiyuki Hino.) a soft science company (Showa 62) and Towbin H. et al: Proc.Natl.Acad.Sci.USA76-4350 (1979), etc. -- the method currently indicated by literature being used conveniently and, Neither in particular the concrete process, nor reagents, conditions, etc. are limited.

[0057]

By using the above-mentioned protein assay, SHP1 protein will be quantified using an antigen-antibody reaction. Therefore, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by a simple mechanism.

[0058]

Next, as the more concrete technique of the above-mentioned mRNA assay, The method of detecting the manifestation of mRNA of SHP1 gene using the polynucleotide which has an overall length, or its part and homology of a base sequence of the SHP1 gene cDNA shown in the array number 3 (refer to drawing 21) is mentioned, More specifically, they are a northern blotting method, a reverse transcription polymerase chain reaction method (RT-PCR), a real-time reverse transcription polymerase chain reaction method (real time RT-PCR), or RNA in. A situ hybridization can be mentioned.

[0059]

The above-mentioned northern blotting method, RT-PCR, real time RT-PCR, And RNA in A method that any method of a situ hybridization is conventionally publicly known. for example," - Molecular cloning"a laboratory manual, Sambrook J., Russell DW., and Cold Spring Harbor Lab Press. (2001). "Current protocols in molecular biology"edited by Ausubel FM et al. and John Wiley & Sons Inc. (2001) etc. -- the method currently indicated by literature. It can use conveniently and neither in particular the concrete process, nor reagents, conditions, etc. are limited.

[0060]

The above-mentioned northern blotting method and RNA in situ Theoretically in hybridization, the overall length of cDNA of SHP1 gene shown in the array number 3 or its part can be used as

a probe. The oligonucleotide which has theoretically the part and homology of cDNA of SHP1 gene shown in the array number 3 also by RT-PCR or real time RT-PCR can be used as a primer. The primer pairs specifically shown in Example 3 mentioned later or Example 4, for example can be used.

[0061]

So, what is necessary is just to detect the manifestation of mRNA of SHP1 gene in mRNA assay using the polynucleotide which has an overall length, or its part and homology of a base sequence of the SHP1 gene cDNA shown in the array number 3.

[0062]

Since SHP1mRNA which is a transcript of SHP1 gene will be quantified by using the above-mentioned mRNA assay, a mechanism simple by using the polynucleotide which has cDNA and homology of SHP1 gene as a probe or a primer -- quickness -- high -- it becomes possible specific and to detect hematopoietic organ tumor cells to high sensitivity.

[0063]

The SHP1 gene-methylation check process in this invention, Especially if it is the method that methylation of the CpG island included in the base sequence of SHP1 gene obtained from a sample sample can be checked, are not limited, but in this embodiment. For example, the method (the expedient top of explanation and restriction enzyme ascertainment are called hereafter) using a methylation sensitivity restriction enzyme including a gene cutting trial stage, a gene amplification trial stage, and the amount check stage of gene amplification can be used conveniently.

[0064]

With the methylation sensitivity restriction enzyme used by this embodiment. When cytosine is included in the base sequence which serves as a recognition object in double stranded DNA and cytosine in this base sequence is methylated, it will not be limited especially if it is a restriction enzyme which cannot cut the double stranded DNA of this base sequence.

[0065]

Specifically as the above-mentioned methylation sensitivity restriction enzyme, HpaII, EagI, or NaeI can be mentioned, for example. Especially, HpaII can be used more preferably. Although it is the endonuclease which HpaII recognizes the base sequence of CCGG and carries out double-stranded-DNA cutting, MspI is known as a restriction enzyme which recognizes the same base sequence and cuts double stranded DNA.

[0066]

As mentioned above, HpaII cannot cut the double stranded DNA of the base sequence of methylated CCGG, but it is not concerned with the existence of methylation, but the base sequence of CCGG is recognized, and MspI can cut double stranded DNA. That is, MspI is a methylation insusceptible restriction enzyme. So, by using HspII and MspI together, it can become possible to use as control for checking cutting of SHP1 gene in a sample sample certainly, and the reliability of the restriction enzyme ascertainment in this embodiment can be further raised so that it may mention later.

[0067]

Thus, in the restriction enzyme ascertainment in this embodiment, it is preferred to use the methylation insusceptible restriction enzyme which recognizes the same base sequence as the methylation sensitivity restriction enzyme to be used as control. Of course, it cannot be overemphasized that the combination of methylation sensitivity and a methylation insusceptible restriction enzyme is not what is limited to above-mentioned HspII-MspI.

[0068]

Next, the check of methylation of SHP1 gene by the SHP1 gene-methylation check process in this embodiment, i.e., restriction enzyme ascertainment, is explained concretely.

[0069]

First, it processes with the above-mentioned methylation sensitivity restriction enzyme which recognizes the base sequence which includes for cytosine the genetic material obtained from said sample sample containing a hematopoietic organ cell as a gene cutting trial stage. In this stage, cutting of SHP1 gene contained by processing of a methylation sensitivity restriction enzyme in a genetic material is tried. That is, if the hematopoietic organ cell contained in said sample sample is only a normal cell, SHP1 gene will be cut, but if hematopoietic organ tumor cells are contained, since CG arrangement is methylated, SHP1 gene will not be cut.

[0070]

From said sample sample, the method of preparing a genetic material can especially use a publicly known method conventionally, and is not limited. The prepared genetic material should just contain SHP1 gene, and other ingredients may be contained unless restriction enzyme processing, PCR, etc. are checked. So, what is necessary is just a mixture of various DNAs or RNA extracted from the hematopoietic organ cell contained in said sample sample, or other cells. It is not limited in particular for processing by a methylation sensitivity restriction enzyme, and what is necessary is just to set up conditions etc. suitably according to the kind of this methylation sensitivity restriction enzyme, the state of the prepared genetic material, etc.

[0071]

Next, PCR is carried out using the primer which amplifies the field which is included in the base sequence of the SHP1 above-mentioned gene as a gene amplification trial stage to the genetic material processed with the above-mentioned methylation sensitivity restriction enzyme, and includes the base sequence by which recognition cutting is carried out in the above-mentioned methylation sensitivity restriction enzyme. In this stage, amplification of only SHP1 gene is tried by carrying out PCR processing of the restriction enzyme treatment object processed with the methylation sensitivity restriction enzyme using the above-mentioned primer. If it is only SHP1 normal gene, since the field inserted into primer pairs is cut, SHP1 gene cannot be amplified, but if SHP1 gene methylated is contained, since the field inserted into the above-mentioned primer pairs is not cut, SHP1 gene will be amplified.

[0072]

What is necessary is just the polynucleotide which amplifies the field which includes the base sequence recognized by the methylation sensitivity restriction enzyme as the above-mentioned primer used in the above-mentioned gene amplification trial stage. So, it is not limited in particular for the design condition of a primer. The primer pairs used by this embodiment fundamentally, Even if there are few fields including the above-mentioned base sequence recognized by the methylation sensitivity restriction enzyme, it is located outside, What is necessary is just a partial base sequence included in the base sequence of SHP1 gene shown in the array number 1 or 2 (refer to drawing 1 - drawing 10 and drawing 11 - drawing 20), or the polynucleotide which has this partial base sequence and complementarity, and it is not limited in particular for that place, size, etc.

[0073]

Next, the quantity of the amplified gene is checked as an amount check stage of gene amplification. In this stage, it is checked whether SHP1 gene has been amplified. If SHP1 gene is amplified, hematopoietic organ tumor cells will be contained in the original sample sample.

[0074]

Although not limited especially as the check method of the existence of SHP1 gene used in the above-mentioned amount check stage of gene amplification, since the technique of checking the amplifying amount of a gene by comparing with a marker using an electrophoresis method is a most common and established technique, it can use preferably. Blotting of the DNA band obtained after electrophoresis may be carried out to a membrane, and it may be detected.

[0075]

Although not limited especially as the check method of the existence of SHP1 gene used in the above-mentioned amount check stage of gene amplification, Since the technique of checking the amplifying amount of a gene using an electrophoresis method is a most common and established technique after reacting using a methylation positivity and methylation negative control DNA simultaneously with a sample sample, it can use preferably. Blotting of the DNA band obtained after electrophoresis may be carried out to a membrane, and it may be detected.

[0076]

SHP1 gene should just be used for the methylation positivity of the SHP1 above-mentioned gene, and methylation negative control DNA, and they are not limited in particular. The DNA solution which has the concentration of the grade which can measure an amplifying amount specifically obtained by processing with a methylation sensitivity restriction enzyme or a methylation insensitive restriction enzyme can be mentioned.

[0077]

It is desirable, when the same sample sample is processed with a methylation insensitive restriction enzyme and it is checked in the amount check stage of gene amplification in parallel to processing by a methylation sensitivity restriction enzyme as control in the SHP1 gene-methylation check process by restriction enzyme ascertainment. That is, it is dramatically preferred to use the restriction enzyme in which the methylation restriction enzyme [ susceptible ] which recognizes the same base sequence is known as a methylation sensitivity restriction enzyme in the above-mentioned gene cutting trial stage. This can raise the certainty of methylation of SHP1 gene by restriction enzyme ascertainment.

[0078]

The SHP1 satellite LOH check process in this invention, Especially if it is the method that the existence of heterozygosity loss (it abbreviates to Loss of heterozygosity and LOH) of SHP1 gene contained in this sample sample can be checked in the sample sample containing a hematopoietic organ cell, are not limited, but specifically, The Microsatellite marker which puts SHP1 gene, Or about gene polymorphism (polymorphism) like the simple nucleotide polymorphism (single nucleotide polymorphism, SNP) which exists the inside of the above-mentioned SHP gene, and near it. The method of checking LOH in the fragmentation analysis using PCR can be used conveniently.

[0079]

About the Microsatellite marker which exists in the both sides of the SHP1 above-mentioned gene, and the gene polymorphism which exists in SHP1 gene and near it. Although not the thing limited especially but what kind of marker may be used, specifically, D12S336 marker and D12S356 marker can be mentioned, for example. The base sequence of these markers is acquired from the Internet genome database (URL:<http://gdbwww.gdb.org/>). Among these markers, D12S356 marker exists in the telomere side, and is in the distance of about 4.4 cM(s) from SHP1 gene. On the other hand, D12S336 marker exists in the centromere side, and is in the distance of about 2.4 cM(s) from SHP1 gene.

[0080]

When checking LOH (heterozygosity loss) of SHP1 gene in a sample sample, the sample sample used by a SHP1 satellite LOH check process should just be a sample sample containing a hematopoietic organ cell. What is necessary is just to conduct Microsatellite analysis which detects at least one overall length of each above-mentioned marker, or its part by an PCR reaction, as shown in Example 6 mentioned later although the concrete method in particular of LOH is not limited. In particular the conditions besides an PCR reaction at this time are not limited, either, and as a primer for PCR, For example, what is necessary is for what is necessary just to be a primer which can detect at least some of D12S336 markers or D12S356 markers, and just to set up conditions relevant also about other conditions suitably.

[0081]

The sample sample used by the SHP1 satellite LOH check process in this invention will not be limited especially if it is a sample sample containing a hematopoietic organ cell. As contrast, the sample sample in particular to be used is not limited, either, the sample obtained after complete hematological remission may be used, and other normal tissue cells may be used.

[0082]

Thus, it becomes possible to detect hematopoietic organ tumor cells more certainly by a simple mechanism by checking LOH of SHP1 gene using gene polymorphism, such as a Microsatellite marker and SNP.

[0083]

Although the example which checked LOH of SHP1 gene using a Microsatellite marker and gene polymorphism is given in this embodiment, If this invention is a method which it is not limited to this and LOH of SHP1 gene can check, it cannot be overemphasized that what kind of method may be used.

[0084]

Next, a desirable example of the detecting method concerning this embodiment is explained more concretely.

[0085]

First, at least SHP1 protein contained by the SHP1 gene-product fixed-quantity process in a sample sample using the technique mentioned above or SHP1mRNA either is quantified. When SHP1 protein quantified in this process is decreasing in number more nearly substantially than a standard or SHP1 gene product is hardly revealed, a possibility that hematopoietic organ tumor cells are contained in the sample sample becomes high.

[0086]

Next, methylation of the CpG island included by said restriction enzyme ascertainment by a SHP1 gene-methylation check process in the base sequence of SHP1 gene in the genetic material prepared from the sample sample is checked. In the following explanation, the example which used said HpaII as a methylation sensitivity restriction enzyme is given. Although the base sequence of CCGG is recognized to have mentioned above, HpaII is preferably used, in order that the methylation insusceptible restriction enzyme MspI may recognize the same base sequence.

[0087]

So, in a gene cutting trial stage, the genetic material obtained from the above-mentioned sample sample is processed by HpaII. Simultaneously, it is desirable when the same genetic material is processed by MspI. By this, positive control that a CCGG base sequence is cut can be obtained.

[0088]

Next, although it shifts to a gene amplification trial stage, in this step, the primer for PCR is previously set up across the recognition site (CCGG) of HpaII/MspI from the base sequence (refer to the array numbers 1 and 2, [drawing 1](#) - [drawing 10](#) and [drawing 11](#) - [drawing 20](#)) of SHP1 gene. The primer pairs specifically shown in Example 1 mentioned later or Example 2, for example are used.

[0089]

Using the above primers, PCR is carried out to the genetic material processed by HpaII, it is the amount check stage of gene amplification, and the amplifying amount of an PCR product is checked, for example by electrophoresis. Since HpaII cannot be cut if SHP1 methylated gene is in a genetic material, the PCR product of the target size is detectable by PCR. On the other hand, without SHP1 methylated gene, DNA is cut by HpaII and an PCR product cannot be detected.

[0090]

Thus, if the above-mentioned restriction enzyme ascertainment is used, after trying cutting of SHP1 gene contained in the genetic material obtained from the sample sample using the methylation sensitivity restriction enzyme and amplifying using PCR further, the amplifying amount of the PCR product acquired can be checked. So, if even a little SHP1 genes are obtained from a sample sample, it is possible to detect methylation of SHP1 gene. Therefore, even if hematopoietic organ tumor cells exist very much in a sample sample only in the minute amount, it is promptly high detection sensitivity, and it becomes possible to detect hematopoietic organ tumor cells on a high unique target moreover.

[0091]

There is that no other process (process) and other stages (step) may be included in the above-mentioned detecting method explained by this embodiment also until it says. For example, in the SHP1 gene-methylation check process, in order to advance a restriction enzyme reaction and an PCR reaction smoothly, the refined stage which refines the genetic material etc. which were obtained may be included.

[0092]

The detection kit for enforcing not only the hematopoietic organ tumor cell detection method mentioned above but this detecting method is contained in this invention. Specifically, the composition containing said SHP1 antibody, said methylation sensitivity restriction enzyme, said each primer, said SHP1 gene positivity, methylation negative control DNA, etc. can be mentioned. It is desirable when it divides into the combination of (1) above-mentioned SHP1 antibody and (2) methylation-sensitivity restriction enzyme, the primer for PCR, and said SHP1 gene positivity and methylation negative control DNA especially, and either [ at least ] (1) or (2) are contained. Whichever of the turn of a SHP1 gene-product fixed-quantity process and a SHP1 gene-methylation check process may be the point.

[0093]

Other various reagents may be contained in the above-mentioned detection kit if needed. For example, at least one side of reagents for a restriction enzyme reaction, such as reagents for an PCR reaction, such as a nucleotide monomer, polymerase, and a buffer, and a buffer, may be contained.

[0094]

The reagent etc. which are used for every process or stage are explained more concretely. First, in a gene product fixed-quantity process, even if it is any of an enzyme-labeled antibody technique and a western blotting method in the case of protein assay, SHP1 antibody and its detecting reagent are used at least. In the case of mRNA assay, they are a RT-PCR assay and real

time. When using a RT-PCR assay, the primer for SHP1cDNA detection and a Taq DNA polymerase reaction reagent are used at least.

[0095]

Next, in the SHP1 gene-methylation check process in this embodiment, in order to check methylation with a methylation sensitivity restriction enzyme, a methylation sensitivity restriction enzyme, methylation insensitive restriction enzymes, and these reaction reagents are first used at least in a gene cutting trial stage. Next, in a gene amplification trial stage, a primer, a Taq DNA polymerase reaction reagent, and SHP1 gene-methylation positive DNA for system studies are used at least. Next, in the amount check stage of gene amplification, the reaction product which used the SHP1 gene-methylation positivity and methylation negative control DNA can be used at least as control of electrophoresis.

[0096]

Thus, in the detection kit concerning this invention, in order to enforce the hematopoietic organ tumor cell detection method mentioned above, desirable drugs, a specimen, etc. are contained. Therefore, the hematopoietic organ tumor cell detection method concerning this invention can be enforced easily and simply by using the above-mentioned detection kit, and it becomes possible to use this invention on industrial levels, such as clinical laboratory test industry and pharmaceutical industry.

[0097]

[Embodiment 2]

It will be as follows if other gestalten of the operation in this invention are explained based on drawing 25 thru/or drawing 47. This invention is not limited to this. The explanation of explanation which overlaps with Embodiment 1 for convenience is omitted suitably.

[0098]

Although the restriction enzyme ascertainment which uses a methylation sensitivity restriction enzyme for a SHP1 gene-methylation check process was used in said Embodiment 1, This invention is not limited to this and the method (the expedient top of explanation and a DNA modification method are called hereafter) of embellishing DNA using a bisulfite including a gene ornamentation stage and a methylation cytosine content judging stage can be conveniently used for it by this embodiment, for example.

[0099]

Cytosine will be changed into uracil if DNA is processed by a bisulfite (Bisulfite). As shown in drawing 25, cytosine is specifically sulfonated by the bisulfite (Sulphonation), Furthermore it is deaminated by hydrolysis (Hydrolytic deamination) and is further changed into uracil by the desulfonation (Alkali desulphonation) under alkali existence. This uracil places and changes to thymine after PCR. On the other hand, the methylated cytosine (5'-methylcytosine) is not changed by a bisulfite. So, in this embodiment, using the difference in the base sequence after this bisulfite processing, the existence of methylation of SHP1 gene is detected so that it may mention later.

[0100]

Next, the check of methylation of SHP1 gene by the SHP1 gene-methylation check process in this embodiment, i.e., a DNA modification method, is explained concretely.

[0101]

First, the genetic material obtained from said sample sample containing a hematopoietic organ cell as a gene ornamentation stage is processed by a bisulfite. In this stage, since only the cytosine which is not methylated is changed into uracil as mentioned above, if bisulfite



processing of the DNA is carried out, as shown in drawing 26, so, the methylated cytosine (M enclosed with a figure middle circle shows) remains with cytosine, for example, but. The cytosine which is not methylated is changed into uracil (U).

[0102]

Especially as a bisulfite used in the above-mentioned gene ornamentation stage, although not limited, sodium bisulfite (it is also called  $\text{Na}_2\text{S}_2\text{O}_5$ , the sodium metabisulfite, sodium disulfite, or sodium pyrosulfite) can be used conveniently, for example. Urea may be used together with a heavy sulfurous acid compound.

[0103]

Next, the existence of cytosine in the base sequence of SHP1 gene contained in the genetic material processed by the bisulfite as a methylation cytosine content judging stage is judged. That cytosine is contained in SHP1 gene in a bisulfite treatment object, the methylated cytosine will be contained in SHP1 gene before processing. So, if cytosine exists, hematopoietic organ tumor cells will be contained in the original sample sample.

[0104]

Although not limited especially as a method of judging the existence of cytosine in the base sequence of SHP1 gene carried out in the above-mentioned methylation cytosine content judging stage, specifically, 1) Which technique can be preferably used at least among the way PCR detects methylation cytosine, the way the determination of the base sequence of a gene detects 2 methylation cytosine, or the method of identifying the base sequence containing 3 methylation cytosine.

[0105]

More specifically, methylation specific PCR (Methylation Specific PCR) can be first mentioned as a way PCR detects 1 methylation cytosine.

[0106]

The above-mentioned methylation specific PCR method is specific to methylated DNA, and sets up as a primer a base sequence including CG arrangement. If the methylated cytosine exists, amplification will become possible by PCR, and SHP1 gene so methylated can be detected.

[0107]

A publicly known method (for example, the method of Proc. Natl. Acad. Sci. USA 93, 9821-9826 (1996), etc. currently indicated by literature) can be conventionally used for the above-mentioned methylation specific PCR method conveniently, and The concrete process and reagents, Conditions in particular are not limited. In the refining processes of DNA, if the primer which could use the method etc. which used the ethanol precipitation method and the Glassbeads method, and carried out the fluorescence label is used, detection of PCR can be made easy.

[0108]

Next, in sequencing of the way, i.e., SHP1 gene, the determination of the base sequence of two genes detects methylation cytosine, a primer is set as the field which does not include CG arrangement, and PCR is carried out. In the PCR product acquired, that (it exists with CG arrangement) by which it is methylated, and the thing (changed into TG arrangement) which is not methylated may be contained. Existence of CG arrangement, i.e., methylation, is considered by carrying out sequencing of this.

[0109]

A method that sequencing of the SHP1 above-mentioned gene is also conventionally publicly known. (For example, the method currently indicated by the literature of Proc. Natl. Acad. Sci. USA 89, 1827-1831 (1992), etc.) can be used conveniently, and neither in particular the concrete

process, nor reagents, conditions, etc. are limited. It is also possible to use the primer which has specific arrangement (CG arrangement is included) for methylated DNA as the above-mentioned primer.

[0110]

Since PCR is used, this method can also detect methylation of SHP1 gene, if even a little SHP1 genes are obtained from a sample sample. Therefore, even if hematopoietic organ tumor cells exist very much in a sample sample only in the minute amount, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by high detection sensitivity. Since concrete arrangement is determined by using sequencing, it also becomes possible to clarify the grade of methylation more.

[0111]

Next, as a method of identifying the base sequence containing 3 cytosine, the Ms-SnuPE method, the bisulfite SSCP method, the methyl light method, a fluorescence dissolution curvilinear analysis method, the COBRA method, etc. can be mentioned.

[0112]

The describing [ above ] Ms-SnuPE (Methylation-sensitive Single Nucleotide Primer Extension) method is a method of carrying out PCR using a specific primer to methylated DNA. However, since the existence of methylation in the field inserted into the primer is not known, the polynucleotide which adjoins CG arrangement to detect is created and it is made to anneal with an PCR product. If  $^{32}\text{P}$ -dCTP is incorporated when DNA is compounded under existence of radioisotope, the cytosine methylated since it is CG arrangement there will exist. Since it is TG arrangement there, it means that methylation was not carried out on the other hand if  $^{32}\text{P}$ -dTTP was incorporated when DNA is compounded.

[0113]

A publicly known method (for example, the method of Nucleic Acids Research 25, 2529-2531 (1997), etc. currently indicated by literature) can be conventionally used for the describing [ above ] Ms-SnuPE method conveniently, Neither in particular the concrete process, nor reagents, conditions, etc. are limited.

[0114]

The describing [ above ] bisulfite SSCP (Bisulfite-SSCP) method does not understand the existence of methylation in the field inserted into the primer, either, although it is the method of carrying out PCR using a specific primer to methylated DNA. Then, electrophoresis of the PCR product is carried out after denaturalizing to single-strand DNA using the SSCP (Single Strand Conformational Polymorphism) method, and the grade of methylation of SHP1 gene is judged from the difference in the mobility of single-strand DNA.

[0115]

The describing [ above ] bisulfite SSCP method can also use conveniently conventionally a publicly known method (for example, the method of Electrophoresis 21, 904-908 (2000), etc. currently indicated by literature), and neither in particular the concrete process, nor reagents, conditions, etc. are limited.

[0116]

To others, the methyl light (Methyl-light) method, the fluorescence dissolution curvilinear analyzing (Fluorescence Melting Curve Analysis) method, etc. are mentioned. No these methods also understand the existence of methylation in the field inserted into the primer, although it is the method of carrying out PCR using a specific primer to methylated DNA. then -- the field which wants to investigate the inside -- methylation -- the quantity of methylation in the above-

mentioned PCR product is judged by creating specific polynucleotide and examining how much [ the PCR product which this methylation specific polynucleotide made the single strand, and ] an annealing (2 chain polymerization) reaction is carried out.

[0117]

The methyl light describing [ above ] method specifically, for example Nucleic Acids Research 28 (8), E32 (2000), etc. -- the method currently indicated by literature -- the above-mentioned fluorescence dissolution curvilinear analysis -- concrete -- Clinical Chemistry 47 and 1183-1189 (2001), etc. -- the method currently indicated by literature can be used conveniently.

[0118]

Since PCR is used, the all directions method mentioned above can detect methylation of SHP1 gene, if even a little SHP1 genes are obtained from a sample sample. Therefore, even if hematopoietic organ tumor cells exist very much in a sample sample only in the minute amount, it becomes possible to detect hematopoietic organ tumor cells on a high unique target by high detection sensitivity.

[0119]

The describing [ above ] COBRA method () [ Combined Bisulfite Restriction Analysis and ] Or if CGCG arrangement has received [ being called Bisulfite PCR followed by restriction analysis etc. and ] methylation, for example, after heavy sulfite treating remains with CGCG arrangement, but. It will be changed into TGTG arrangement if not methylated. Then, by using the restriction enzyme etc. which cut only the above-mentioned CGCG arrangement, the band pattern on electrophoretic gel can be analyzed, and the existence of methylation of SHP1 gene can be judged and quantified.

[0120]

The describing [ above ] COBRA method can also use conveniently conventionally a publicly known method (for example, the method of Nucleic Acids Research 25-2532-2534 (1997) etc. currently indicated by literature), Neither in particular the concrete process, nor reagents, conditions, etc. are limited. Of course, the advantage by PCR mentioned above is not only acquired, but [ since PCR is used also by this method, ] since restriction enzyme processing and electrophoresis are used, if even the analysis of a band pattern is clarified, there is an advantage that methylation of SHP1 gene can be checked easily.

[0121]

Thus, by the DNA modification method in this embodiment, although PCR is used in the methylation cytosine content judging stage, the designing method of a primer used by this PCR is explained below.

[0122]

As mentioned above, if bisulfite processing of the DNA is carried out, cytosine will be changed into uracil, but the methylated cytosine is saved, without being changed. Here, the cytosine which may receive methylation by intracellular is cytosine (C) of CG arrangement (5'-CG-3') located in a line with CG from the 5' arrangement side. Therefore, all cytosine other than the above-mentioned CG arrangement will be changed into thymine (T) by bisulfite processing. Then, all the CG arrangement changes the base sequence of SHP1 gene as what received methylation, and sets up a primer. Uracil in DNA will be recognized as thymine and will be replaced by thymine by PCR.

[0123]

First, the conditions about the DNA strand which plans a primer are set up. In the base sequence of SHP1 gene, either a sense strand or an antisense strand assumes the arrangement from which

all cytosine in other base sequences was changed into thymine noting that only the above-mentioned CG arrangement receives methylation.

[0124]

. The base sequence shown in drawing 27 - drawing 36, and the array number 5 specifically corresponds to the sense strand of the genomic DNA (wild type) of SHP1 gene shown in drawing 1 - drawing 10, and the array number 1. It is a base sequence (it is hereafter considered as sense strand conversion arrangement on [ of explanation ] expedient) after bisulfite processing. . Correspond for the base sequence shown in drawing 37 - drawing 46, and the array number 6 to consider it as the antisense strand of the genomic DNA (wild type) of SHP1 gene shown in drawing 11 - drawing 20, and the array number 2. It is a base sequence (it is hereafter considered as antisense strand conversion arrangement on [ of explanation ] expedient) after bisulfite processing. These sense strand conversion arrangement and antisense strand conversion arrangement become less complementary by bisulfite processing.

[0125]

The base sequence of drawing 27 - drawing 36, the array number 5 and drawing 37 - drawing 46, and the array number 6, When it is assumed that CG arrangement is methylated 100%, it is a base sequence as what received bisulfite processing, and since it is not thought that 100% of methylation arises in a cell actually, it illustrates as a base sequence as a possibility that it can detect in this invention.

[0126]

And a forward primer (FW primer) and a reverse primer (RV primer) are created to the (I) above-mentioned sense strand conversion arrangement, or FW primer and RV primer are created to the (II) above-mentioned antisense strand conversion arrangement. In this case, primer arrangement differs also at the same place, respectively.

[0127]

Next, the conditions about the field which plans a primer are set up. (i) In order to amplify what created the primer to the base sequence including CG arrangement, or was (ii)-methylated in order to amplify only methylated DNA by PCR directly, and the thing which is not carried out by PCR fair, create a primer to the arrangement which does not include CG field. When it is (ii), the method of sequencing or others is enforced later and methylation is judged.

[0128]

Therefore, there are four kinds of designing methods which multiplied condition (i) and condition (ii) about condition (I) about the above-mentioned DNA strand and condition (II), and a field in the design of the primer used by a DNA modification method.

[0129]

Here, in (i), if the place of the primer has received methylation with sufficient convenience, it will be detected, but when only the adjacent area instead of a place have received methylation, it becomes undetectable although methylation exists. Then, methylation of SHP1 gene is certainly detectable by authorizing the existence of the methylation in the field which was not concerned with the existence of methylation but was surrounded by each primer after amplification by PCR like (ii), i.e., CG arrangement. Therefore, the information of not only the place of the primer for detection but a gene sequence itself becomes important for the judgment of methylation of SHP1 gene in this embodiment.

[0130]

If CG arrangement is not methylated, will be changed into TG arrangement by bisulfite processing, but. The primer (Unmethylated primer) created to a base sequence including this TG

arrangement can be used as control proving existence of DNA which has not received methylation. When bisulfite processing is insufficient, SHP1 gene of the wild type from which cytosine is not changed into uracil will mix. Then, the primer (Wild type primer) which has a wild type base sequence can be used as control of whether bisulfite processing was made thoroughly enough.

[0131]

In the methylation cytosine content judging stage mentioned above. The method same to the check of the gene amplified by PCR as the amount check stage of gene amplification in said Embodiment 1, For example, by comparing with a marker using an electrophoresis method, the amplifying amount of a gene is checked or the technique of having \*\*\*\*\*ed enough blotting of the DNA band further obtained after electrophoresis to the membrane, and carrying out it is mentioned. Of course, it is not limited to these techniques, and a publicly known technique can be conventionally used conveniently also about the above-mentioned electrophoresis method or the method of blotting, and it is not limited in particular.

[0132]

As long as it puts in another way, the amount check stage of gene amplification as well as the case where it is based on the restriction enzyme ascertainment in said Embodiment 1 may be included also by the SHP1 gene-methylation check process by the DNA modification method in this embodiment.

[0133]

Next, a desirable example of the detecting method concerning this embodiment is explained more concretely.

[0134]

First, either [ at least ] SHP1 protein contained by the SHP1 gene-product fixed-quantity process in a sample sample using the technique mentioned above or SHP1mRNA is quantified. When SHP1 protein quantified in this process is decreasing in number more nearly substantially than a standard or SHP1 gene product is hardly revealed, a possibility that hematopoietic organ tumor cells are contained in the sample sample becomes high.

[0135]

Next, methylation of the CpG island included by said DNA modification method by a SHP1 gene-methylation check process in the base sequence of SHP1 gene in the genetic material prepared from the sample sample is checked. specifically, the genetic material obtained from the above-mentioned sample sample is come out of and processed in a gene ornamentation stage using sodium bisulfite.

[0136]

Next, although it shifts to a gene amplification trial stage, in this step, the primer for PCR is set up based on the designing method of the primer mentioned above.

[0137]

By methylation specific PCR, as shown in drawing 47 (a), specifically, suppose that CG arrangement of wild type DNA has methylation 100% supposing wild type DNA (for figure Nakagami, the bottom is an antisense strand at a sense strand) of 23 base pairs. When bisulfite processing is carried out, it becomes in this case, less complementary in a sense strand and an antisense strand, as shown in drawing 47 (b). Then, as shown in drawing 47 (c) or (d), FW primer and RV primer are created to a sense strand or an antisense strand.

[0138]

In above-mentioned methylation specific PCR, the primer pairs specifically shown in Example 4

mentioned later or Example 5, for example are used as a primer for PCR. Using the above primers, methylation specific PCR is carried out to the genetic material processed with sodium bisulfite, for example, the amplifying amount of an PCR product is checked by electrophoresis. [0139]

Thus, if the describing [ above ] DNA modification method is used and the genetic material obtained from the sample sample using the bisulfite will be processed, cytosine in a base sequence will be changed into uracil, but the methylated cytosine is not changed. Therefore, methylation of SHP1 gene is detectable only by judging whether cytosine is contained in the base sequence of SHP1 gene after a gene ornamentation stage. Therefore, it becomes possible quick by a simple mechanism and to detect hematopoietic organ tumor cells on a high unique target.

[0140]

Next, either [ at least ] SHP1 protein contained by the SHP1 gene-product fixed-quantity process in a sample sample using the technique mentioned above or SHP1mRNA is quantified. When SHP1 gene products quantified in this process are decreasing in number more nearly substantially than a standard or are hardly revealed, a possibility that hematopoietic organ tumor cells are contained in the sample sample becomes high.

[0141]

Like the detecting method of said Embodiment 1, there is that no other process (process) and other stages (step) may be included in the above-mentioned detecting method explained by this embodiment also until it says.

[0142]

The detection kit for enforcing not only the hematopoietic organ tumor cell detection method mentioned above but this detecting method is contained in this invention. The composition which specifically contains the bisulfite refined to the gene processing level, said primer, and said SHP1 antibody can be mentioned. In the detection kit concerning this invention, the above-mentioned bisulfite, a primer, and SHP1 antibody That is, (1) above-mentioned SHP1 antibody and (2) bisulfites, The primer for a judgment of the existence of cytosine in the base sequence of SHP1 gene contained in the genetic material processed by this bisulfite, And when it divides into the primer for PCR which detects the overall length of a base sequence, or its part of SHP1 gene cDNA shown in the (3) array number 3, it is preferred that any at least one of (1), (2), and (3) is included.

[0143]

The probe for Northern blotting which has an overall length, or its part and homology of a base sequence of the SHP1 gene cDNA shown in the array number 3 in the above-mentioned detection kit, Or the marker for electrophoresis using the methylation positivity of a restriction enzyme and SHP1 gene and methylation negative control DNA which recognize the base sequence containing cytosine may be included, At least one side of reagents for a restriction enzyme reaction, such as reagents for an PCR reaction, such as a nucleotide monomer, polymerase, and a buffer, and a buffer, may be contained.

[0144]

The reagent etc. which are used for every process or stage are explained more concretely. First, in a gene product fixed-quantity process, since it is the same as that of what was mentioned as the example by said Embodiment 1, the explanation is omitted.

[0145]

Next, in the SHP1 gene-methylation check process in this embodiment, in order to check

methylation by bisulfite processing, reagents, such as various bisulfites, are first used at least in a gene ornamentation stage. Next, in a methylation cytosine content judging stage, when using how PCR detects methylation cytosine, methylation arrangement specific primers and a TaqDNA polymerase reaction reagent are used at least. moreover -- the way the determination of the base sequence of a gene detects methylation cytosine, or the method of recognizing the base sequence containing cytosine -- each -- publicly known reagents are used according to a concrete method.

[0146]

Thus, in order to enforce the hematopoietic organ tumor cell detection method mentioned above like [ the detection kit concerning this embodiment ] the detection kit of said Embodiment 1, desirable drugs, a specimen, etc. are contained. Therefore, the hematopoietic organ tumor cell detection method concerning this invention can be enforced easily and simply by using the above-mentioned detection kit, and it becomes possible to use this invention on industrial levels, such as clinical laboratory test industry and pharmaceutical industry.

[0147]

This invention is not what is limited to each embodiment mentioned above, It cannot be overemphasized that it is contained in the technical scope of this invention also about the embodiment obtained by embodiment which various change is possible and is different in the range shown in the claim combining suitably the technical means indicated, respectively.

[0148]

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## EXAMPLE

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[Example]

Hereafter, the concrete example of this invention is described based on drawing 48 thru/or drawing 52. This invention is not limited to this.

[0149]

[Example 1]

In accordance with Towbin H. et al:Proc.Natl.Acad.Sci.USA76-4350 and the method currently indicated by (1979), Western blotting was carried out using the sample sample containing a spontaneous killer cell lymphoma. #SH-PTP1(D-11):sc7289 (product made from Santa Cruz Biotechnology Inc.) was used as SHP1 antibody (a SHP1 gene-product fixed-quantity process and protein assay).

[0150]

Then, it shifted to the SHP1 gene-methylation check process. First, the genetic material prepared from the above-mentioned sample sample was processed in 37 \*\* 4 hours, using HpaII as a methylation sensitivity restriction enzyme (gene cutting trial stage).

[0151]

Next, the genetic material processed by HpaII was amplified by PCR (gene amplification trial stage). The Pullar \*-\*\*\*\* used at this time was taken as the combination of primer REP-S1 of 19 bases shown in the array number 7 and drawing 48 (a), and primer REP-AS1 of 20 bases shown in the array number 8 and drawing 48 (b). When these primer pairs are used, as shown in the array number 9 and drawing 48 (c), the base sequence of 126 bases from 7441 bases in the arrangement (refer to the array number 1 and drawing 1 - drawing 10) of the sense strand of SHP1 gene to 7566 bases is detected.

[0152]

"# (number)" in the parenthesis in drawing 48 (c) shows the position of the base in the sense strand of the SHP1 above-mentioned gene, and the underline part shows the correspondence position of primer REP-S1 and REP-AS1, and the position of the recognition cleavage site of HpaII. Primer REP-AS1 is designed to the arrangement of the antisense strand in the field of the underline part of above-mentioned REP-AS1.

[0153]

Then, after carrying out electrophoresis by agarose gel, blotting of the obtained DNA band was carried out to the nylon membrane, and amplification of SHP1 gene was checked (the amount check process of gene amplification).

[0154]

Next, Towbin H. et al: Western blotting was carried out in accordance with Proc.Natl.Acad.Sci.USA76-4350 and the method currently indicated by (1979). #SH-PTP1(D-11):sc7289 (product made from Santa Cruz Biotechnology Inc.) was used as SHP1 antibody (a SHP1 gene-product fixed-quantity process and protein assay).

[0155]

The hematopoietic organ tumor cells in a sample sample were detected from the result of the above-mentioned SHP1 gene-methylation check process and a SHP1 gene-product fixed-quantity process.

[0156]

[Example 2]

Primer REP-S2 of 21 bases shown in the array number 10 and drawing 49 (a) as primer pairs in a gene amplification trial stage, The existence of the hematopoietic organ tumor cells in a sample sample was detected like said Example 1 except having used the combination of primer REP-AS2 of 21 bases shown in the array number 11 and drawing 49 (b).

[0157]

When the above-mentioned primer pairs are used, as shown in the array number 12 and drawing 49 (c), the base sequence of 227 bases from 6858 bases in the arrangement (refer to the array number 1 and drawing 1 - drawing 10) of the sense strand of SHP1 gene to 7084 bases can be detected.

[0158]

"# (number)" in the parenthesis in drawing 49 (c) shows the position of the base in the sense strand of the SHP1 above-mentioned gene, and the underline part shows the correspondence position of primer REP-S2 and REP-AS2, and the position of the recognition cleavage site of HpaII. Primer REP-AS2 is designed to the arrangement of the antisense strand in the field of the underline part of above-mentioned REP-AS2.

[0159]

[Example 3]

The existence of the hematopoietic organ tumor cells in a sample sample was examined like said Example 1 except having carried out the SHP1 gene-product fixed-quantity process using the mRNA assay by RT-PCR.

[0160]

That is, after preparing RNA intracellular [ all the ] from said sample sample, reverse transcription was carried out with reverse transcriptase. Then, it amplified by PCR using SHP1 specific primer pairs. As the above-mentioned SHP1 specific primer pairs, the combination of primer SHP-PF1 of 23 bases shown in the array number 13 and drawing 50 (a) and primer SHP-



PR1 of 25 bases shown in the array number 14 and drawing 50 (b) was used.

[0161]

[Example 4]

The existence of the hematopoietic organ tumor cells in a sample sample was examined like said Example 3 (namely, said Example 1) except having carried out the SHP1 gene-product fixed-quantity process using the mRNA assay by real time RT-PCR. As the above-mentioned SHP1 specific primer pairs, primer SHP-LR1 of 20 bases shown in primer SHP-LF1, and the array number 16 and drawing 51 (b) of 20 bases shown in the array number 15 and drawing 51 (a) was used.

[0162]

[Example 5]

Proc. Natl. Acad. Sci. USA 93 and 9821-9826 (1996), Except having carried out the SHP1 gene-methylation check process using methylation specific PCR in accordance with the method currently indicated, The existence of the hematopoietic organ tumor cells in a sample sample was examined like said Example 1. Sodium bisulfite was used as a bisulfite.

[0163]

As primer pairs in above-mentioned methylation specific PCR, the combination of primer MF2 of 24 bases shown in the array number 17 and drawing 52 (a) and primer MR2 of 21 bases shown in the array number 18 and drawing 52 (b) can be used. When these primer pairs are used, as shown in the array number 19 and drawing 52 (c), the base sequence of 159 bases from 7037 bases in the arrangement (refer to the array number 1 and drawing 1 - drawing 10) of the sense strand of SHP1 gene to 7195 bases can be detected.

[0164]

"# (number)" in the parenthesis in drawing 52 (c) shows the position of the base in the sense strand of the SHP1 above-mentioned gene, and the underline part shows the correspondence position of the primers MF2 and MR2. However, since each above-mentioned primer is designed detect only DNA methylated, the base sequence differs from the base sequence of the above-mentioned underline part for a while. Primer MR2 is designed to the arrangement of the antisense strand in the field of the underline part of above-mentioned MR2.

[0165]

[Example 6]

As a sample sample, the bone marrow (BM) sample for diagnosis and the ALL (acute lymphoblastic leukemia) patient's peripheral blood (PB) sample were used. BM sample obtained from the ALL patient contained the blast cell by at least 70% of ratio. The control sample to these sample sample was obtained after the complete hematological remission attained by the chemotherapy.

[0166]

Microsatellite analysis was conducted using the above-mentioned sample sample. the PCR reaction at this time -- the primer by the side of 5' -- 5' -- a label being carried out by - iodoacetamidfluorescein and the system of reaction, It was considered as each dNTP of each primer of 10pmol, the genomic DNA of 40ng, a 1xPCR buffer, and 200microM, and the system of 20microl containing Taq DNAPolymerase of 0.5unit. The acquired PCR product is covered over ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA), It analyzed by Genescan Analysis software ver 3.7 (Applied Biosystems).

[0167]

As a result, as shown in drawing 53 (a) - (b), it turned out that the existence of LOH of SHP1

gene can be checked with D12S336 marker and D12S356 marker. In the result of this example, LOH was observed in 15 examples (79%) with D12S356 marker by the side of a telomere among these markers among 19 cases from which the significant result was obtained. In D12S336 marker by the side of centromere, LOH was observed in six examples (38%) among 16 cases.

[0168]

Any result of the example of the above was fully able to detect hematopoietic organ tumor cells from the sample sample. So, even if this invention did not use two or more modalities together, it turned out that hematopoietic organ tumor cells are easily and promptly detectable.

[0169]

[Effect of the Invention]

As mentioned above, the hematopoietic organ tumor cell detection method concerning this invention, The SHP1 gene-product fixed-quantity process of quantifying either [ at least ] SHP1 specific protein or its mRNA into the hematopoietic organ cell contained in the sample sample containing a hematopoietic organ cell, The SHP1 gene-methylation check process which checks the methylation of the CpG island included in the base sequence of SHP1 gene which encodes the SHP1 above-mentioned protein acquired from the above-mentioned sample sample, It is a method including the SHP1 gene LOH check process which checks the existence of heterozygosity loss (LOH) of SHP1 gene contained in the above-mentioned sample sample.

[0170]

As a desirable example of the hematopoietic organ tumor cell detection kit concerning this invention, (1) The methylation sensitivity restriction enzyme which recognizes the base sequence which contains in a hematopoietic organ cell SHP1 antibody which uses protein tyrosine phosphatase SHP1 specific protein as an antigen, and (2) cytosine, The primer for PCR which amplifies the field which is included in the base sequence of SHP1 gene and includes the base sequence recognized by the above-mentioned methylation sensitivity restriction enzyme, In the composition which contains at least one side the methylation positivity of the SHP1 above-mentioned gene, and among methylation negative control DNAs. Or SHP1 antibody which uses protein tyrosine phosphatase SHP1 protein specific into (1) hematopoietic organ cell as an antigen and the bisulfite refined to (2) gene processing level, The primer for a judgment of the existence of cytosine in the base sequence of SHP1 gene contained in the genetic material processed by this bisulfite, And it is used in order to detect hematopoietic organ tumor cells from the sample sample containing the composition containing any at least one of the primers for PCR which detect the overall length of a base sequence, or its part of SHP1 gene cDNA shown in the (3) array number 3, or a hematopoietic organ cell, The composition containing the primer for PCR which detects at least one overall length of two Microsatellite markers which put protein tyrosine phosphatase SHP1 specific gene between a hematopoietic organ cell, or its part can be mentioned.

[0171]

According to the method or composition of this invention, SHP1 gene expression can be judged using four-fold marker by the maximum called loss of ornamentation and mRNA of gene DNA, protein, and allele. namely, one hematopoietic organ tumor cell called a SHP1 gene-expression fall -- since a specific phenomenon can be checked in four steps, hematopoietic organ tumor cells are detectable by very high singularity. Therefore, hematopoietic organ tumor cells are easily and promptly detectable from a small amount of sample samples containing a hematopoietic organ cell by using this invention.

[0172]

So, if the high sensitivity detection system of the malignant lymphoma and leukemia in this invention is used, The monitoring after the early detection of the hematopoietic organ tumor by a general mass screening, diagnosis, and a therapy and the early detection of a recurrence become possible, and it also becomes possible to use this invention for prediction etc. of the crisis risk in blood relationship persons, such as a family who showed the symptoms of these diseases. As a result, the effect of becoming possible to use this invention on industrial levels, such as clinical laboratory test industry and pharmaceutical industry, is done so.  
[0173]

**<110> Japan Science and Technology Corporation**

**<120> Hematopoietic malignant cell-d  
lignant cell-detection kit**

**<130> Y2002-P331**

<160> 19

<170> Patent In Ver. 2.1

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<220>

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| actaccaccc  | cctccaggtg  | tcttaggcac  | gct |
| actccctctt  | gcagggtgtcc | ttaagttttgc | tcg |
| tcctgagatc  | gccagcctgt  | caggcaagct  | gaa |
| gccaccccat  | gggcctgctg  | ctggtggcag  | cgt |
| ttgtgccact  | gccagggggag | gaaaggccct  | gat |
| gacttagcct  | tgggtgtcagt | ctcttgcgga  | cct |
| tccctctgcc  | tttccaggcc  | ccatccccct  | gaa |
| cctaaccctg  | ccccaggggcc | taaccctacc  | tga |
| tgagaggctg  | gagtgggtcc  | ctcagcgccc  | tgg |
| ccttcctctga | ggaactgggc  | tgttagggat  | ttt |
| gagagggtttc | ccccatttgt  | tgctcttctc  | cag |
| tacccaatac  | cccgccgctc  | tgtcagcttg  | agc |
| ctctcccggg  | ggaaggcggc  | cctgggaccag | cag |
| gctgcaggga  | agctggccgc  | tgtggggcgg  | ctc |

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| t a c a g a g a g a   | t g c t g t c c c g | t g g g t a a g t c | c c g      |            |            |      |
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| a g g c g a g a g g   | a t t g c t t g a g | c c c a g g a g t t | t g a      |            |            |      |
| c t g t c t c t a c   | a a a a a a t a a g | a a a t t a g c t g | g g t      |            |            |      |
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| c c c g t g g c a g   | a g g a c c c t a g | t g g a g c g g a g | g c a      |            |            |      |
| a g c t a g g a t g   | t t g a a a g t g a | a a a c c t g a c g | a g a      |            |            |      |
| c a c t t t t g g g a | g g c c g a a g g g | g g a a g a t t g c | t t g      |            |            |      |
| c a a c a t a g a g   | a g a c c c c a t c | t c t a t t a a a a | a a a      |            |            |      |
| t g g t a g t c c t   | a g c a g t t t g g | g a g g c t g a g g | t g g      |            |            |      |
| g a c c a c c c t g   | g g c a a c a t a g | g g a g a g a c c t | c a t      |            |            |      |

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| cgctgtctgc  | tgcagtatgg  | ttgttggggg   | aaa        |            |            |      |
| cgtgtttttac | ccaccttcct  | actcactagc   | ttg        |            |            |      |
| tgagtccttag | tttctgtttc  | taaaattggg   | tga        |            |            |      |
| gaggattaat  | agtataatgt  | aaaagctggc   | agc        |            |            |      |
| tcacatcagt  | atttgggaaa  | tattgttaag   | ctc        |            |            |      |
| agagcagttc  | cagaactttc  | tacagattat   | ttt        |            |            |      |
| tcttcttgta  | tcaccattga  | tcttgatctg   | tat        |            |            |      |
| ggagttttcac | tctgttgccc  | aggctggagt   | gcg        |            |            |      |
| tccacctcct  | gagaagctgg  | gattacaggc   | tag        |            |            |      |
| agctgggtctc | gaactcctga  | cctcaagtga   | tcc        |            |            |      |

|                     |                     |                     |            |            |            |      |
|---------------------|---------------------|---------------------|------------|------------|------------|------|
| gattacaggt          | giaagtcact          | gcgcccagct          | glatitttat | ittttgagac | agggtctcac | 4500 |
| t c t g t c a c c c | a g g c c g g a t t | a c a g t g g c a c | a a c      |            |            |      |
| c a g g c t c a a g | c g a t c c t c c c | a t c t c a g t c t | c c c      |            |            |      |
| a c c a c a c c t g | g c t a a a t t t t | g t a t t t t t t g | t a g      |            |            |      |
| c t g c t c t c a a | a c t t g g t g t c | a a g t a a t c c a | c c a      |            |            |      |
| a c a g g c g t g a | g c c a c t g c g c | c t g g c c t t g a | t c t      |            |            |      |
| a a t a t t t t t t | t c t t c t g a a t | t a t c a g g c a t | t t a      |            |            |      |
| t t g t t t c c g c | c c a t a a g a a a | a t g g g g a a a a | t g a      |            |            |      |
| c t a a a t g a g a | t c g t g t a t g t | g a a a g t g a t c | t g c      |            |            |      |
| a g g t a g t t t t | t t a t t t t c c t | g c c a a a g g a t | a g c      |            |            |      |
| g t c t c t t g g c | t t g a c a c c c t | t c a g a g g a a t | t c c      |            |            |      |
| g c c t a g t t a g | t t t c t t c t g g | g t g c c g a g t t | a a t      |            |            |      |
| t g g a a g g a g t | g t c t t g g g g c | c a g g g t g c a g | t g g      |            |            |      |
| a c t t c g t g t g | t g g t c a g a t t | t a t g t t c c a t | g c g      |            |            |      |
| g t g t a g g t g g | a c a t g g a t g a | t g a g g t g t g t | g t g      |            |            |      |
| c g t a t g a c a a | g c a g g c t g t g | t g t g t a g g a c | c a g      |            |            |      |
| g c t a t g g a c t | g a c g a g c t t g | t t t g t t g a a c | a c t      |            |            |      |
| t c t g a a c a c t | c c g a g a t g a g | c g a g a g c g c c | a g c      |            |            |      |
| c a t g t g c t c t | t c t t g c t c c t | t g g c g t t g g g | c g t      |            |            |      |
| g g g g t g g t g t | c c a a g c t g a a | g g g g t a g t c a | a a g      |            |            |      |
| a g a t t c c c c a | g g g c a c c a g t | g a g a g a a g a a | a a c      |            |            |      |
| a a g a a a a g t g | g a a a a g c c t t | t t t t g g g g g a | a a a      |            |            |      |
| a t a a t g t a g t | t a t c a t g g g a | a a a t t a g a c t | t g t      |            |            |      |
| c t g a a g c a g a | a c a t g c a t a a | t g t t c a t a a a | t a t      |            |            |      |
| t t t t t t t t t t | g a a a t a g a g t | c t t a c t g t g t | t g c      |            |            |      |
| g g c t c g c t g c | a a c c t c g g c t | t c c t g g g t t c | a a g      |            |            |      |
| a g c t g g g a t t | a c a g g c g c c c | a c c a c c a t g c | c c a      |            |            |      |
| a g g g g g t t t c | a c c a t g t t g g | c c a g g a t g g t | c t c      |            |            |      |
| c t t g g c c t c c | c a a a g t g c t g | g g a t t a c a g g | c g t      |            |            |      |

tttatacaca cccaigcaaa cagcatccag atagagacaa agagccttcc ctgtacccta 6240

|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| a a a g t t t c c c | a g a a a t t g t t | c c c a g t t a g c | a t a |
| c a t c a t a t a a | c a t t c a a a a a | g g t a t g t a g a | g a a |
| c a g c c a c c c a | g t t t c c c t c c | c t a g g g g a a g | c c a |
| g t t g a g c t g c | t t t t c c t c g t | t t t g g t t t g g | c g g |
| g t a g g c a g c a | t c a t a t a c c t | t a g t g t t t a g | g g c |
| t c a g c c a t g g | t g a g g a c c t t | g t c c c c c a g c | c c c |
| c t g g g g c a g g | g c a g a g g c c t | a g g g a c a a g a | a t t |
| t c a g g g t c a t | g t t g t c c a t c | c c t c t g c c a c | a g t |
| a g a g g a g g g c | a c c c t t c t c t | c t t g c a a g c a | t t g |
| t c c c a t g g c a | g c c c c t t t g g | a c a a g g a g g c | t c t |
| g c t g g t g t a t | a g g a g t t c a a | a g c a c t g g c t | t t g |
| g g c a c t g c a g | c t g a c t c a c t | g a t g g a c t c a | g g c |
| t t c c t t g t c t | g t a a a a t g a t | a a a g a t a g c c | c c t |
| a t c a g a c a a g | g c a t g t g a a c | g c c a t t a t a g | c a c |
| c g a t g a c a g t | t g t c a c c g c c | a t c a t t g t t a | t t a |
| g c a g c t g g t g | g a g g a g g g a g | a g a t g c c g t g | g g a |
| t a t c t g g g c c | t g g a g t g t g c | a a g g c a c a c a | t g t |
| g t g c a a t g c c | a t g c t c c t g a | g c c t t t g a t t | g c a |
| a c c c c c a g t g | c c a c c c t g c t | c t g c t t c t c t | t c c |
| a a g t g a g t t c | c c c c a a g g g g | t c g g c c g c g c | c t c |
| c a g g c c a g t g | g a g t g g c a g c | c c c a g a a c t g | g g a |
| a c t g g g a g c t | g c a t c t g a g g | c t t a g t c c c t | g a g |
| c t c c t c a t t c | c c t g c g c c c c | c t t c c t c t c c | g g a |
| t g c c a c c c a c | g g t a g a c a g g | a g g c a a g g g t | g c c |
| c c c t g c c t g g | g c c g c c c a g g | t g g t t t c a c c | g a g |
| t g c t c a a g g g | c c g a g g t g t c | c a c g g t a g c t | t c c |
| g t g a c t t c t c | g c t c t c c g t c | a g g t a g g t g g | g c c |
| t c t c t t g t g c | c a t c c a g g c c | c t g a a c c a c t | c a t |



ccccgtctgt tccctigccc ccaacccccca cactccccat cctgtctgt gccacccat 7980

|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| g c c c a t g t g t | g c c c c c a c c c | a g g a c c t c a g | c c g |
| c a c c g a c t g g | c c t c a c c g c c | t g g t g c c c t g | c a g |
| g a t c c a g a a c | t c a g g g g a t t | t c t a t g a c c t | g t a |
| a g a g c t g g t g | g a g t a c t a c a | c t c a g c a g c a | g g g |
| c a t c c a c c t c | a a g t a c c c g c | t g a a c t g c t c | c g a |
| c a c c c c c g c c | a t t c c c a a g c | a g g g a t g a g c | c g g |
| g g g a g a c t g g | c a g c c g g c g c | t g c c t a c c c t | c c a |
| g c t c t c a a t g | t c c c t c c t c c | c t g c t g t c c t | g g g |
| a c c c t t t c c a | c c t a a c c c c g | a g g a a g c c a c | a g a |
| c t g g c c g c t g | c a a c c c a g g t | c c c a c t g g a g | a c a |
| t c g t g c a g g c | c a g c t c t g t t | g t t a g a a a g c | t c t |
| c t c c g t c t g c | c c c t c a c c c c | a g c a c a t g t t | a g g |
| g a t g g g g a t g | a a t g c t t g c c | a a g a c a c t t g | a t g |
| g g t c t g t c c t | g t g g g g t c a a | a t a g g t c t c c | g g c |
| g t g a a g t g t t | c a c c t g t g t a | a a g t g t c t c a | c g c |
| g c a t t t c c t t | c c t g t g g c c t | c c c c g a c t c c | t c c |
| g g c t g g g g g c | t c t g a a t g c t | c c t c a t g a c a | c c a |
| t g c c a g a t c c | c c t t a g a g t a | a a g g g c a g c g | g a a |
| c c t g g g c c a a | g c c g a c t t g c | c c t t g c c g t g | g a t |
| a t g a t c g g g a | a c c t t g c t c c | t g c c a g c t t g | c a g |
| g c a t c a a t a t | t t t t g g t c a a | g g c a c t g a t t | g a a |
| a g c c c t g t g g | c c c a c c t g g g | a g g c c t c c t t | t c c |
| t c t c t c t g t g | a g c c t c a c a t | g g c t g g c t c c | g t g |
| c c g c a a c a c t | c a g g g g g c t t | t t g g c a c c g a | g a c |
| c c t t g c c t c c | a g c c a g g a g a | g g a g g a c g g g | c t g |
| g c a g g g c c c c | a g g a g g c c c c | t g c a g a g g a g | g c t |
| a g a g a g a g a a | g g a a g g g a g g | g c a g t g c c g g | g g c |
| t g g a g g c c c t | t t t g g g t g a c | c c g t c c c a g g | a g c |

gtgagaggct ctctccca ggctctgctg tctctctgc ctgtctgtg cgcctctcc 9720

|                     |                     |                       |       |
|---------------------|---------------------|-----------------------|-------|
| t c t g c g a g a a | t t t g c a t c t g | t c c c t c g g t g   | g c t |
| t t g c a t g g a g | a c t t c c t c a t | c c t g g g g c c t   | g a g |
| c c t g g g g t c c | t a g c c t g t c c | c c a g g c g g t g   | g g c |
| t c t g g g g g t c | t c t c g g c t g g | a g t c a c c t c c   | g g g |
| g g t c c t c c c c | t c c t t c c c c c | c a t c c c t g c g   | g t t |
| c c t g g g c t g a | g g a a a c c t c a | c a a c c t c a c t   | t c t |
| t t t t t t c c a t | c a c g t g g t t t | c c t g t g g g g c   | t g g |
| g g a a a g g g g t | g t g c t t c g g g | g a a a g g g c t t   | a g t |
| a a a t c c g t t t | g a a c c c t g g g | c t c c c c t t c a   | g t g |
| c t a c a c c a c t | c t t t c c c c a g | t g g g g t t g t c   | t t c |
| t c c g c c t t c c | t t g t g a c t t g | a g t c t g t g t g   | t c c |
| c t c g g t c t g c | g t t t c t c t t t | g c c t c t g g t c   | t c t |
| a g a t t c a t c c | t t a g c t t c t c | t c c t c c a a a t   | a t t |
| c a g a g g t g g g | c t c t g g g t t c | g a a g c c c g g t   | t a g |
| c t g g g a g g t c | g a g g c t g c a g | a g a g c t g t a a   | c c g |
| g a g c t c t g g a | a g c t t g c c c t | a g a g t c a g t c   | a a g |
| g c g t c a g t t t | c c t c a t c t a t | a a a a t g g g g g   | t a a |
| t g a g a g a c c t | a a a t g a g g t g | g t g g a t t t g g   | a a g |
| a g g t g c t t g a | t t t c c g g c c c | c t c t c t g t g a   | a t g |
| c t g g g t c t t a | c c t t c c c t g a | c g c t g c c t t c   | t c t |
| g g g c a g g c a g | a g a c g c t g c t | g c a g g c c a a g   | g g c |
| a g c c t c a g c c | a g c c t g g a g a | c t t c g t g c t t   | t c t |
| c c a g g c t c c c | c g c t c a g g g t | c a c c c a c a t c   | a a g |
| g g c g g g g g a g | c c t c t g c t g a | g g c t c c t g t c   | t g t |
| c t g c c t g g g c | t t g a a t t c a a | g g c t g g g g a c   | c c a |
| g c c t a a t t t g | g c t c c c c c c a | g g g t g g a c g c   | t a c |
| a g c c t c a c g g | a c c t g g t g g a | g c a t t t t c a a g | a a g |
| t t t g t c t a c c | t g c g g c a g g t | c a g g g g t g g g   | c c c |

|             |             |             |            |            |           |       |
|-------------|-------------|-------------|------------|------------|-----------|-------|
| ccccccagat  | gigagcttct  | gggatctctg  | agttgctgac | tctcgtctct | ccccacccc | 11460 |
| agccgtacta  | tgccacgagg  | gtgaatgcgg  | ctg        |            |           |       |
| acaagaagca  | ggagtcggag  | gatacagcca  | agg        |            |           |       |
| atggtgggga  | ccggcagggc  | tgggggcagct | gag        |            |           |       |
| cggacacctt  | ccccctccttg | cccacctcttg | ctc        |            |           |       |
| atggatgccc  | tcttttgggag | ctgatgctca  | ttt        |            |           |       |
| agcaggagggt | gaagaacttg  | caccagcgtc  | tgg        |            |           |       |
| agaaccgcta  | caagaacatt  | ctccccctgtg | agc        |            |           |       |
| accgccccctg | ccccagctgc  | ctccccctcat | ctc        |            |           |       |
| ggggccatctc | cccacacccc  | ccacagagcc  | tcc        |            |           |       |
| ccagaagtgc  | ctccccacca  | ccagcaggca  | gggt       |            |           |       |
| actccctcac  | tccctccata  | cagatgatcc  | ccc        |            |           |       |
| gcctcatggc  | ttctgagacc  | agaatggcct  | gtt        |            |           |       |
| gtgagtcacct | ggctaacca   | gaccatctctg | cct        |            |           |       |
| agccgagtga  | tcctgcaggg  | acggggacagt | aac        |            |           |       |
| aactacatca  | aggtcagcag  | tgtggggccac | gtg        |            |           |       |
| cctgtctgggt | gggggggacc  | tagatccaga  | gac        |            |           |       |
| tgcatggggtg | agggtggcag  | tgggttcagg  | cct        |            |           |       |
| gggggtgcgtc | tctccacgct  | tgcgtccaga  | acc        |            |           |       |
| agacctacat  | cgccagccag  | ggttgtcttg  | agg        |            |           |       |
| cgtggcaggga | gaacagccgt  | gtcatcgtca  | tga        |            |           |       |
| tagggcgccc  | ccccctcccc  | gcataccgccc | ccg        |            |           |       |
| gagcagtcag  | atgccagggc  | agaaaagggat | ctc        |            |           |       |
| aaactgaggg  | ctagtgacaa  | agtcctcgact | aca        |            |           |       |
| atccctgggc  | tcttctgagc  | tccagaccca  | gggt       |            |           |       |
| ctgccccacc  | tgtctgcatac | caggccccctc | ctg        |            |           |       |
| agtcctgcccc | ttaccctgca  | ggctccccctt | aca        |            |           |       |
| gatcccatcc  | gtgacacaaa  | ctggggtcaag | ttc        |            |           |       |
| tcctgggtcac | ctttggggata | aagtcgcact  | cta        |            |           |       |

|             |              |              |            |            |            |       |
|-------------|--------------|--------------|------------|------------|------------|-------|
| tcctctgac   | ccgcacgctt   | ctcttgaagg   | ctcaccgccc | ccagcagccc | cagctctttc | 13200 |
| agggttcccag | ccctttcttttg | cacaaagctca  | ttt        |            |            |       |
| tatctctgccc | tggcagatgc   | ctcgttttttg  | aag        |            |            |       |
| gaatccagggt | cttggtttcct  | ccagggacctta | gag        |            |            |       |
| tcctcagcgc  | ggtgtctccc   | ccgggtcacct  | gtc        |            |            |       |
| acagactggg  | tgttattttgt  | gtctgtgaag   | ctg        |            |            |       |
| gcctgcccctg | gcaacgttttg  | ttgaatgaca   | aac        |            |            |       |
| gcctcaccac  | ctgtttgggtgg | ttgatcttgag  | acg        |            |            |       |
| agcccatccg  | tccatccaac   | aaatgttttgg  | gcc        |            |            |       |
| ggacctggga  | tggggccacag  | tggccctgctc  | tgt        |            |            |       |
| ccagaacaaaa | tgcgtcccat   | actgggcccgga | ggt        |            |            |       |
| ctctgtgacc  | aaactgcggggg | agcatgacac   | aac        |            |            |       |
| ctccccgctg  | gacaaatgtga  | gtggcccccca  | cgc        |            |            |       |
| ggacttggttc | tcctctcttgg  | tcggggtagggg | tga        |            |            |       |
| gggggcactga | ccctatgtcc   | tcgggcttaggg | gag        |            |            |       |
| agtacctgag  | ctgggcccgac  | catgggggtcc  | cca        |            |            |       |
| tggaccagat  | caaccagcgg   | caggaaagtc   | tgc        |            |            |       |
| gcagggtgagg | atgataatcc   | tgatggtagt   | agt        |            |            |       |
| tgccatgagc  | tgttataagc   | aataataaacg  | tta        |            |            |       |
| cccccggtt   | ctcctgggtc   | ccctcatggc   | tcc        |            |            |       |
| ccagcccccac | tttggccctc   | tggcctgtggg  | tat        |            |            |       |
| catctcgccc  | aaccctgccca  | aatacacagagg | agg        |            |            |       |
| ccaagctagt  | caggggcaagg  | ccggggcaggc  | acc        |            |            |       |
| cgcctttctct | cgagggtccca  | ttctgtttggt  | ttc        |            |            |       |
| tgctccccat  | tcctcctctt   | tttccatcgg   | tag        |            |            |       |
| ctgcccctctc | tcccagcttc   | cccagggcagt  | gcc        |            |            |       |
| gatggggtgat | gcttcttttg   | ggctgcacat   | aac        |            |            |       |
| tgatcaggag  | acctctggta   | agggtgcagag  | gtg        |            |            |       |
| acagggtgagc | ccactgagct   | ggccctggccct | ggg        |            |            |       |

|                     |                     |                     |            |            |            |       |
|---------------------|---------------------|---------------------|------------|------------|------------|-------|
| cciccgcit           | ccagctgtgt          | ggctctggac          | aaattactta | acttttctaa | ccctcagctt | 14940 |
| c c t c a t c t g t | a a a a t c a g g a | t c t c a g g g t t | g t c      |            |            |       |
| g t g g c t g g a a | t t c c g t c a g c | c c t c a a a a a c | t g g      |            |            |       |
| t c a g g c a g a g | a a t a g g g g a a | t g g g a a c c t g | c c t      |            |            |       |
| t g g a c c c c a g | g c c t g c g a c g | g c c t c t g g c t | t c c      |            |            |       |
| t g g g a c a g g g | c a a g t c g g c t | g a a t c t a g a g | g t g      |            |            |       |
| c t c t g t c c t g | t g c t c t c t c a | g g g a c a g g c c | c a t      |            |            |       |
| g c c a c a c a c a | c a t t c a c a c a | c t t c t t g a a a | g c c      |            |            |       |
| a a g g a a g t g g | g t g t g g g g g g | t t a t t t t t g a | c a a      |            |            |       |
| t c a g g g c a t c | a g c t t g c t g g | g c t c a g c t g a | g g g      |            |            |       |
| t t g c c c a g g g | c t g g g a a a g g | a g a g a a a c t t | c c t      |            |            |       |
| c c c t g t g c c c | c c g c a c c c t g | c t g t c t c a g g | g c t      |            |            |       |
| g g a a a a g g g a | a g t g a a g c c a | t g c t g a g a g a | c g c      |            |            |       |
| g a g g g c t c a g | g g t a c c t g g g | a g c c g g c a g g | a c a      |            |            |       |
| t c c g g g g t g g | g g g c a g c c a c | t c a c t a g g a g | t g a      |            |            |       |
| a a g g a t g g t g | g c a g c t g g g g | a g c c a g c g t c | a g c      |            |            |       |
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| ctcttttgtct  | ctatctggat  | gctgttttgca   | tgg        |            |           |       |
| cgcagtggtct  | cacgcctgta  | atcccagcac    | ttt        |            |           |       |
| gtcaggagat   | cgagaccatc  | ctggccaaca    | tgg        |            |           |       |
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| caggagaatc   | acttgaacc   | aggaagccga    | ggt        |            |           |       |
| actccagcct   | ggcaaacacag | taagactcta    | ttt        |            |           |       |
| ttgtgtgctt   | aatattttatg | aacatttatgc   | atg        |            |           |       |
| ttttaagccc   | aacaaagtcta | atttttcccat   | gat        |            |           |       |
| aaacatcaat   | gtttttccccc | aaaaaaaggct   | ttt        |            |           |       |
| atttggtttct  | ggtttttcttc | tctcactggt    | gcc        |            |           |       |
| gcatggtcca   | gcttttgacta | cccccttcagc   | ttg        |            |           |       |
| gcggaacccc   | cacgcccac   | gccaaaggagc   | aag        |            |           |       |
| gcgggacacc   | cgctggcgcct | ctcgcctcatc   | tcg        |            |           |       |
| ggcactgagc   | aagtgttcaa  | caaacacaagct  | cgt        |            |           |       |
| agtacagctt   | cctgggtccta | cacacacagc    | ctg        |            |           |       |
| cacagtcaca   | gcacacacac  | ctcatcatcc    | atg        |            |           |       |
| gcacatcccc   | acgcatggaa  | cataaatctg    | acc        |            |           |       |

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| gtacagcttc   | tgctatcctt  | tggcaggga    | ata       |          |          |       |
| gtgtgggggtt  | tgcagatcac  | tttcacatac   | acg       |          |          |       |
| tgatgtagga   | atcatttttcc | ccatttttctt  | atg       |          |          |       |
| aattacaaga   | gtaaatgcct  | gataatttcag  | aag       |          |          |       |
| gataaaaagta  | gagatcaagg  | ccaggcgccag  | tgg       |          |          |       |
| ggggggtgagg  | ctgggtggatt | acttgacacc   | aag       |          |          |       |
| aaacccctgtc  | tctacaaaaa  | atacaaaaatt  | tag       |          |          |       |
| cccagggtact  | tggggagactg | agatggggagg  | atc       |          |          |       |
| agtgagccat   | ggttgtgccca | ctgtaatccg   | gcc       |          |          |       |
| aaaaataaaa   | atacagctgg  | gcgcagtgac   | tta       |          |          |       |
| ccgagggtggg  | aggatcactt  | gagggtcagga  | gtt       |          |          |       |
| ccccatctct   | actagcctgt  | aatcccagct   | tct       |          |          |       |
| gatcatgccca  | ccgcactcca  | gacctggggcaa | cag       |          |          |       |
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| gacctgacaaa  | tgagcttaac  | aatattttccc  | aaa       |          |          |       |
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| gccaagggtc   | acaagctagt  | gagtaggaag   | gtg       |          |          |       |
| gacctgggtgcc | ctttcccca   | acaaccatac   | tgc       |          |          |       |
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| tcaaggccag   | gctgagaaac  | caaggatgaa   | atc       |          |          |       |
| cctggcagga   | aaatgaccac  | agacgggatc   | agg       |          |          |       |
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| c a g g c t g g a g | t g c a g t g a c g | c a a t c t c g g c | t c a      |            |             |       |
| c a a t t c t c c t | g c c t c a g c c t | c c c t a g t a g c | t g g      |            |             |       |
| g c c t a a t t t t | t t t t t t g t a t | c t t t a g t t g a | g a c      |            |             |       |
| t c t t g a a c t c | c t g a c c t c g t | g a t c c a c c t g | c c t      |            |             |       |
| g c t t g a g c c a | c c a t g c c c a g | c c g g t t g t c a | c t t      |            |             |       |
| c t t t t t c t t t | t t c t t t c c t t | t c t t c t t t c g | a c t      |            |             |       |
| t t c t t t t c t t | t c t t t c c t t c | c t t c c t t t t c | t t t      |            |             |       |
| c t t c t t t c t c | t t t t t c t t t c | t t c c t t c c c t | t c c      |            |             |       |
| t t t t t t t g a c | a c a g a a t c t t | g c t t t a t c a c | c c a      |            |             |       |
| t c a c t g c a g c | g t c g a c c t c c | t t g g c t c a g g | t g a      |            |             |       |
| t g a g a c c a c a | g g t g c a t g c c | a c t a c a t c c a | g c t      |            |             |       |
| t c g t a g t a g a | g a t g a g g t c t | c t c c c t a t g t | t g c      |            |             |       |
| a a g t g a t c c t | c c c a c c t c a g | c c t c c c a a a c | t g c      |            |             |       |
| t a c c c a g t a t | t t t t t t t t a a | t a g a g a t g g g | g t c      |            |             |       |
| a c t c c t g a g c | t c a a g c a a t c | t t c c c c c t t c | g g c      |            |             |       |
| g c g c c a c c t c | a t c t c g t c a g | g t t t t c a c t t | t c a      |            |             |       |
| t g c t g t g g c c | c t g c c t c c g c | t c c a c t a g g g | t c c      |            |             |       |
| t g t t t g t t c c | c a c a t t a g g g | c t t t g t g c t c | a c c      |            |             |       |
| c t t g a t c a c t | a c g t c a c a t g | g c c a g c t c c t | c t t      |            |             |       |
| g t c a c c t c c t | c g g g a a g g c c | t c c c t g a t c c | c t c      |            |             |       |
| g t c a c t t g c t | a g c a t a t g a a | g c t g g t t c a t | t t t      |            |             |       |
| g t c t c a c t c t | g t c g c c c a g g | c t g g t g t g c a | g t g      |            |             |       |
| a c c t c c c a g g | c t c a a g c a a g | c c t c t c a c c t | t a g      |            |             |       |
| t g t g c c a c c a | c a c c c a g c t a | a t t t c t t a t t | t t t      |            |             |       |
| c c a g g c t g g t | c t c a a a c t c c | t g g g c t c a a g | c a a      |            |             |       |
| t g g g a t c a c a | g g t g t g a a c c | a c t a c a c c c g | g c c      |            |             |       |
| t c t g a g a a t c | t g c a a t g a t c | c a a t t t a t t g | g g t      |            |             |       |



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|                     |                     |                     |       |
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| g c a g g g g a a a | g g g a a g t g a a | a g g a a a c a a a | g a a |
| g t g c g g a g a a | g t t g g c t g g a | c g t g g g a g g g | c c t |
| t g a g g t g t t g | t g a g c t a c c c | a g g g g g a c a g | g t c |
| a g a g g c t g g a | a g t a g a a a t g | c a a t g g a g a g | a a c |
| t t a a a g a g g g | g g c a g a g g a g | c a t g a g t g a g | c c g |
| a a g t t c a t a g | g g a c c g g a a a | c a g g c g c a g g | g c a |
| c g t c g g a g t g | a g c a t c a a c a | a a g c c c t c c c | t c c |
| c c g a t g g t g c | c c g g g a c t t a | c c c a c g g g a c | a g c |
| g g c g g a g g g c | c g c a g g g g g c | g g g g c t g a g c | c a g |
| c c a g g t g a g g | a a g a a c c g c t | c a g t a a t c a g | c c a |
| c a a t g g c a c t | g c a g g g a c a a | a c c c t g g a c t | a a t |
| g g g g c t g g c c | c g a g a c c g c c | c a c a g c g g c c | a g c |
| a g c a g g c c c g | c c t g c t g g t c | c a g g g c c g c c | t t c |
| c t c c a c c t g g | a g c t c a a g c t | g a c a g a g c g g | c g g |
| a g t a a c c c t g | g c t g a g g a a g | a g c a a c c a a t | g g g |
| a g g g c c t a a g | g a a a a t c c c t | a a c a g c c c a g | t t c |
| g g c c c a c c c a | c c c a g g g c g c | t g a g g g a c c c | a c t |
| g g g g a g g a g c | c t c a g g t a g g | g t t a g g c c c t | g g g |
| g g g a g g a g c t | g t t c a g g g g g | a t g g g g c c t g | g a a |
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| t t g t c t g g a a | c a t c a a g g c c | t t t c c t c c c c | t g g |
| a g g a g g c g g c | c a c g c t g c c a | c c a g c a g c a g | g c c |
| g a a a c a g c g c | c t t c a g c t t g | c c t g a c a g g c | t g g |
| a c t t g a c c a a | g c g a g c a a a c | t t a a g g a c a c | c t g |
| t c c t a a g g a c | c a g c g t g c c t | a a g a c a c c t g | g a g |
| c c c c c a c t g g | c t g g g c a g c t | g t t t g c c a a a | c a g |
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g a c t g t g a c a   t t g a c a t c c a   g a a g a c c a t c   c a g
g t g c a g a c g g   a g g c g c a g t a   c a a g t t c a t c   t a c
a c t a a g a a g a   a g c t g g a g g t   c c t g c a g t c g   c a g
a t c a c c t a t c   c c c c a g c c a t   g a a g a a t g c c   c a t
c a c a a g g a g g   a t g t g t a t g a   g a a c c t g c a c   a c t
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Gln Gln Gly Val Leu Gln Asp Arg Asp  
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Tyr Pro Leu Asn Cys Ser Asp Pro Thr  
100 105

His Met Ser Gly Gly Gln Ala Glu Thr  
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Pro Trp Thr Phe Leu Val Arg Glu Ser  
130 135

Val Leu Ser Val Leu Ser Asp Gln Pro  
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Leu Arg Val Thr His Ile Lys Val Met  
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Val Gly Gly Leu Glu Thr Phe Asp Ser  
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Phe Lys Lys Thr Gly Ile Glu Glu Ala  
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Arg Gln Pro Tyr Tyr Ala Thr Arg Val Asn Ala Ala Asp Ile Glu Asn  
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Arg Val Leu Glu Leu Asn Lys Lys Gln  
225 230

Ala Gly Phe Trp Glu Glu Phe Glu Ser  
245

Asn Leu His Gln Arg Leu Glu Gly Gln  
260 265

Asn Arg Tyr Lys Asn Ile Leu Pro Phe  
275 280

Gln Gly Arg Asp Ser Asn Ile Pro Gly  
290 295

Tyr Ile Lys Asn Gln Leu Leu Gly Pro  
305 310

Ile Ala Ser Gln Gly Cys Leu Glu Ala  
325

Met Ala Trp Gln Glu Asn Ser Arg Val  
340 345

Val Glu Lys Gly Arg Asn Lys Cys Val  
355 360 365

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gln | Arg | Ala | Tyr | Gly | Pro | Tyr | Ser |
|     | 370 |     |     |     |     | 375 |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Thr | Thr | Glu | Tyr | Lys | Leu | Arg | Thr |
| 385 |     |     |     |     | 390 |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Asp | Leu | Ile | Arg | Glu | Ile | Trp |
|     |     |     |     | 405 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Asp | His | Gly | Val | Pro | Ser | Glu | Pro |
|     |     |     | 420 |     |     |     |     | 425 |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Asp | Gln | Ile | Asn | Gln | Arg | Gln | Glu |
|     |     | 435 |     |     |     |     | 440 |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Ile | Val | His | Cys | Ser | Ala | Gly | Ile |
|     | 450 |     |     |     |     | 455 |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ile | Asp | Met | Leu | Met | Glu | Asn | Ile |
| 465 |     |     |     |     | 470 |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ile | Asp | Ile | Gln | Lys | Thr | Ile | Gln |
|     |     |     |     | 485 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Met | Val | Gln | Thr | Glu | Ala | Gln | Tyr |
|     |     |     | 500 |     |     |     |     | 505 |

Ala Gln Phe Ile Glu Thr Thr Lys Lys Lys Leu Glu Val Leu Gln Ser  
515 520

Gln Lys Gly Gln Glu Ser Glu Tyr Gly  
530 535

Met Lys Asn Ala His Ala Lys Ala Ser  
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Glu Asp Val Tyr Glu Asn Leu His Thr  
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|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| a t t a t t a t t t | t t t t t a g g t g | t t t t a g g t a c | g t t |
| a t t t t t t t t t | g t a g g t g t t t | t t a a g t t t g t | t c g |
| t t t t g a g a t c | g t t a g t t t g t | t a g g t a a g t t | g a a |
| g t t a t t t t a t | g g g t t t g t t g | t t g g t g g t a g | c g t |
| t t g t g t t a t t | g t t a g g g g a g | g a a a g g t t t t | g a t |
| g a t t t a g t t t | t g g t g t t a g t | t t t t t g c g g a | t t t |
| t t t t t t t g t t | t t t t t a g g t t | t t a t t t t t t t | g a a |
| t t t a a t t t t g | t t t t a g g g t t | t a a t t t t a t t | t g a |
| t g a g a g g t t g | g a g t g g g t t t | t t t a g c g t t t | t g g |
| t t t t t t t t g a | g g a a t t g g g t | t g t t a g g g a t | t t t |
| g a g a g g t t t t | t t t t a t t g g t | t g t t t t t t t t | t a g |
| t a t t t a a t a t | t t c g t c g t t t | t g t t a g t t t g | a g t |
| t t t t t t c g g g | g g a a g g c g g t | t t t g g a t t a g | t a g |
| g t t g t a g g g a | a g t t g g t c g t | t g t g g g c g g t | t t c |
| t t t t g g a g a t | t a t t a g t t t a | g g g t t t g t t t | t t g |
| c g a g g a g g a a | g t g g t t g a t t | a t t g a g c g g t | t t t |
| t a g t t g t g t c | g t t g g t t t a g | t t t c g t t t t t | t g c |
| t a t a g a g a g a | t g t t g t t t c g | t g g g t a a g t t | t c g |
| t a g t t t t g g a | g g g a g g g a g g | g t t t t g t t g a | t g t |
| c g a t t t g t c g | t t g t t t t g c g | t t t g t t t t c g | g t t |
| g t g a g g a t t t | t c g g t t t a t t | t a t g t t t t t t | t g t |
| a g t g t g t a t t | t g t t t t t t t t | a t t g t a t t t t | t a t |
| t t t t t g t t t a | g g a t t t g t t t | t t t t g g g t a g | t t t |
| g t t a t t c g c g | a a g g t t t t t t | t a c g t t t a g t | t a a |
| t t g g t t t t a t | t t t t t t t g t t | t t t t t t t a t t | t t t |
| g t a c g t g t t g | a g t a t t t a t t | a t g t a t t a g g | t g t |
| g a a t a a g a t a | g a t a t g g t t t | t t g t t t t t a c | g g a |
| t c g a a t a a a t | a a t t t a a t a a | a t t g g a t t a t | t g t |



tagatagittt tggicgggtg tagtggittt tatttgtgat tttagtattg tgggaggttg 1860

|             |               |              |      |
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| ttgtttttat  | aaaaaaaaataag | aaatttagttg  | ggt  |
| ttatggagag  | gttaagggtga   | gaggttttgtt  | tga  |
| gatgattgta  | ttattgtata    | ttagttttggg  | cga  |
| aaaaaaaaaaa | gaaaaatgaat   | tagtttttata  | tgt  |
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| ggatgaggag  | gaaggaggagt   | tggtttatgtg  | acg  |
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| ttcgtggtag  | aggatttttag   | tggagcggag   | gta  |
| agttaggatg  | ttgaaagtga    | aaattttgacg  | aga  |
| tatttttggga | ggtcgaaggg    | ggaagatttgt  | ttg  |
| taatatagag  | agattttatt    | tttatttaaaa  | aaa  |
| tggtagtttt  | agtagttttgg   | gaggtttgagg  | tgg  |
| gattatttttg | ggtaatatag    | ggagagagattt | tatt |
| tatttaataaa | tagtttggaatg  | tagtggtatg   | tatt |
| aggtaggagg  | attattttgag   | ttaaggagggt  | cga  |
| gtatttttagt | ttgggtgata    | aagtaagattt  | ttg  |
| gaaggaaaga  | aggaaaggga    | ggaagaaaga   | aaa  |
| cgagaaagaa  | gaaagaaaag    | gaagggaaggga | aag  |
| aagtgatatt  | tagtcgaaag    | aagaaaggga   | aga  |
| gaaaaaagaa  | aaagtgataa    | tcgggttgggt  | atg  |
| ttgggagggtc | gaggtaggtg    | gattacgagg   | tta  |
| ggtgaaatttt | tgtttttaatt   | aaagatatata  | aaa  |
| atttgtaggt  | tttagttatt    | agggagggttg  | agg  |
| ggaggttgta  | gtgagtcgag    | attgcgttat   | tgt  |
| ttttattttta | aaaaaaaaaaa   | aaaaagaaaaa  | gaa  |
| ggcgagtttg  | tgggtgggtg    | gttttttttagt | ttt  |
| ttgttttttgt | tttagtgtat    | attttgtttat  | tgt  |

|                     |                       |                     |           |            |           |      |
|---------------------|-----------------------|---------------------|-----------|------------|-----------|------|
| gttttttggg          | tttgatttc             | gtttgtgggt          | attttttgt | taggtagttt | ggtaggttt | 3600 |
| t t t t t g g t g t | a g a t t t t a t t   | t t t g g t t t t t | t a g     |            |           |      |
| g g g t t t t t a t | t t t t t a g a a t   | a a t t t t g t t t | t a g     |            |           |      |
| t g t t t a t g t g | g a t t t t g t g c   | g t g t t a t t t t | t t t     |            |           |      |
| g t a t t t t t t t | t t t t a t t t t t   | t a t t a t g g g t | t g t     |            |           |      |
| c g t t g t t t g t | t g t a g t a t g g   | t t g t t g g g g g | a a a     |            |           |      |
| c g t g t t t t a t | t t a t t t t t t t   | a t t t a t t a g t | t t g     |            |           |      |
| t g a g t t t t a g | t t t t t g t t t t   | t a a a a t t g g g | t g a     |            |           |      |
| g a g g a t t a a t | a g t a t a a t g t   | a a a a g t t g g t | a g t     |            |           |      |
| t t a t a t t a g t | a t t t g g g a a a   | t a t t g t t a a g | t t t     |            |           |      |
| a g a g t a g t t t | t a g a a t t t t t   | t a t a g a t t a t | t t t     |            |           |      |
| t t t t t t t g t a | t t a t t a t t g a   | t t t t g a t t t g | t a t     |            |           |      |
| g g a g t t t t a t | t t t g t t g t t t   | a g g t t g g a g t | g c g     |            |           |      |
| t t t a t t t t t t | g a g a a g t t g g   | g a t t a t a g g t | t a g     |            |           |      |
| a g t t g g t t t c | g a a t t t t t t g a | t t t t a a g t g a | t t t     |            |           |      |
| g a t t a t a g g t | g t a a g t t a t t   | g c g t t t a g t t | g t a     |            |           |      |
| t t t g t t a t t t | a g g t c g g a t t   | a t a g t g g t a t | a a t     |            |           |      |
| t a g g t t t a a g | c g a t t t t t t t   | a t t t t a g t t t | t t t     |            |           |      |
| a t t a t a t t t g | g t t a a a t t t t   | g t a t t t t t t g | t a g     |            |           |      |
| t t g t t t t t a a | a t t t g g t g t t   | a a g t a a t t t a | t t a     |            |           |      |
| a t a g g c g t g a | g t t a t t g c g t   | t t g g t t t t g a | t t t     |            |           |      |
| a a t a t t t t t t | t t t t t t g a a t   | t a t t a g g t a t | t t a     |            |           |      |
| t t g t t t t t g t | t t a t a a g a a a   | a t g g g g a a a a | t g a     |            |           |      |
| t t a a a t g a g a | t c g t g t a t g t   | g a a a g t g a t t | t g t     |            |           |      |
| a g g t a g t t t t | t t a t t t t t t t   | g t t a a a g g a t | a g t     |            |           |      |
| g t t t t t t g g t | t t g a t a t t t t   | t t a g a g g a a t | t t t     |            |           |      |
| g t t t a g t t a g | t t t t t t t t g g   | g t g t c g a g t t | a a t     |            |           |      |
| t g g a a g g a g t | g t t t t g g g g t   | t a g g g t g t a g | t g g     |            |           |      |
| a t t t c g t g t g | t g g t t a g a t t   | t a t g t t t t a t | g c g     |            |           |      |

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|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| c g t a t g a t a a | g t a g g t t g t g | t g t g t a g g a t | t a g |
| g t t a t g g a t t | g a c g a g t t t g | t t t g t t g a a t | a t t |
| t t t g a a t a t t | t c g a g a t g a g | c g a g a g c g t t | a g c |
| t a t g t g t t t t | t t t t g t t t t t | t g g c g t t g g g | c g t |
| g g g g t g g t g t | t t a a g t t g a a | g g g g t a g t t a | a a g |
| a g a t t t t t t a | g g g t a t t a g t | g a g a g a a g a a | a a t |
| a a g a a a a g t g | g a a a a g t t t t | t t t t g g g g g a | a a a |
| a t a a t g t a g t | t a t t a t g g g a | a a a t t a g a t t | t g t |
| t t g a a g t a g a | a t a t g t a t a a | t g t t t a t a a a | t a t |
| t t t t t t t t t t | g a a a t a g a g t | t t t a t t g t g t | t g t |
| g g t t c g t t g t | a a t t t t g g t t | t t t t g g g t t t | a a g |
| a g t t g g g a t t | a t a g g c g t t t | a t t a t t a t g t | t t a |
| a g g g g g t t t t | a t t a t g t t g g | t t a g g a t g g t | t t t |
| t t t g g t t t t t | t a a a g t g t t g | g g a t t a t a g g | c g t |
| t t t a t a t a t a | t t t a t g t a a a | t a g t a t t t a g | a t a |
| a a a g t t t t t t | a g a a a t t g t t | t t t a g t t a g t | a t a |
| t a t t a t a t a a | t a t t t a a a a a | g g t a t g t a g a | g a a |
| t a g t t a t t t a | g t t t t t t t t t | t t a g g g g a a g | t t a |
| g t t g a g t t g t | t t t t t t t c g t | t t t g g t t t g g | c g g |
| g t a g g t a g t a | t t a t a t a t t t | t a g t g t t t a g | g g t |
| t t a g t t a t g g | t g a g g a t t t t | g t t t t t t a g t | t t t |
| t t g g g g t a g g | g t a g a g g t t t | a g g g a t a a g a | a t t |
| t t a g g g t t a t | g t t g t t t a t t | t t t t t g t t a t | a g t |
| a g a g g a g g g t | a t t t t t t t t t | t t t g t a a g t a | t t g |
| t t t t a t g g t a | g t t t t t t t g g | a t a a g g a g g t | t t t |
| g t t g g t g t a t | a g g a g t t t a a | a g t a t t g g t t | t t g |
| g g t a t t g t a g | t t g a t t t a t t | g a t g g a t t t a | g g t |
| t t t t t t g t t t | g t a a a a t g a t | a a a g a t a g t t | t t t |

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|                     |                       |                       |       |
|---------------------|-----------------------|-----------------------|-------|
| c g a t g a t a g t | t g t t a t c g t t   | a t t a t t g t t a   | t t a |
| g t a g t t g g t g | g a g g a g g g a g   | a g a t g t c g t g   | g g a |
| t a t t t g g g t t | t g g a g t g t g t   | a a g g t a t a t a   | t g t |
| g t g t a a t g t t | a t g t t t t t g a   | g t t t t t g a t t   | g t a |
| a t t t t t a g t g | t t a t t t t t g t t | t t g t t t t t t t t | t t t |
| a a g t g a g t t t | t t t t a a g g g g   | t c g g t c g c g t   | t t t |
| t a g g t t a g t g | g a g t g g t a g t   | t t t a g a a t t g   | g g a |
| a t t g g g a g t t | g t a t t t g a g g   | t t t a g t t t t t t | g a g |
| t t t t t t a t t t | t t t g c g t t t t t | t t t t t t t t t t c | g g a |
| t g t t a t t t a c | g g t a g a t a g g   | a g g t a a g g g t   | g t t |
| t t t t g t t t g g | g t c g t t t a g g   | t g g t t t t a t c   | g a g |
| t g t t t a a g g g | t c g a g g t g t t   | t a c g g t a g t t   | t t t |
| g t g a t t t t t c | g t t t t t c g t t   | a g g t a g g t g g   | g t t |
| t t t t t t g t g t | t a t t t a g g t t   | t t g a a t t a t t   | t a t |
| t t t c g t t t g t | t t t t t t g t t t   | t t a a t t t t t a   | t a t |
| g t t t a t g t g t | g t t t t t a t t t   | a g g a t t t t a g   | t c g |
| t a t c g a t t g g | t t t t a t c g t t   | t g g t g t t t t g   | t a g |
| g a t t t a g a a t | t t a g g g g a t t   | t t t a t g a t t t   | g t a |
| a g a g t t g g t g | g a g t a t t a t a   | t t t a g t a g t a   | g g g |
| t a t t t a t t t t | a a g t a t t c g t   | t g a a t t g t t t   | c g a |
| t a t t t t c g t t | a t t t t t a a g t   | a g g g a t g a g t   | c g g |
| g g g a g a t t g g | t a g t c g g c g t   | t g t t t a t t t t t | t t a |
| g t t t t t a a t g | t t t t t t t t t t t | t t g t t g t t t t t | g g g |
| a t t t t t t t t a | t t t a a t t t c g   | a g g a a g t t a t   | a g a |
| t t g g t c g t t g | t a a t t t a g g t   | t t t a t t g g a g   | a t a |
| t c g t g t a g g t | t a g t t t t g t t   | g t t a g a a a g t   | t t t |
| t t t c g t t t g t | t t t t t a t t t t t | a g t a t a t g t t   | a g g |
| g a t g g g g a t g | a a t g t t t g t t   | a a g a t a t t t g   | a t g |

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|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| g t g a a g t g t t | t a t t t g t g t a | a a g t g t t t t a | c g t |
| g t a t t t t t t t | t t t g t g g t t t | t t t c g a t t t t | t t t |
| g g t t g g g g g t | t t t g a a t g t t | t t t t a t g a t a | t t a |
| t g t t a g a t t t | t t t t a g a g t a | a a g g g t a g c g | g a a |
| t t t g g g t t a a | g t c g a t t t g t | t t t t g t c g t g | g a t |
| a t g a t c g g g a | a t t t t g t t t t | t g t t a g t t t g | t a g |
| g t a t t a a t a t | t t t t g g t t a a | g g t a t t g a t t | g a a |
| a g t t t t g t g g | t t t a t t t g g g | a g g t t t t t t t | t t t |
| t t t t t t t g t g | a g t t t t a t a t | g g t t g g t t t c | g t g |
| t c g t a a t a t t | t a g g g g g t t t | t t g g t a t c g a | g a t |
| t t t t g t t t t t | a g t t a g g a g a | g g a g g a c g g g | t t g |
| g t a g g g t t t t | a g g a g g t t t t | t g t a g a g g a g | g t t |
| a g a g a g a g a a | g g a a g g g a g g | g t a g t g t c g g | g g c |
| t g g a g g t t t t | t t t g g g t g a t | t c g t t t t a g g | a g t |
| g t g a g a g g t t | t t t t t t t t t a | g g t t t t g t t g | t g t |
| t t t g c g a g a a | t t t g t a t t t g | t t t t t c g g t g | g t t |
| t t g t a t g g a g | a t t t t t t t a t | t t t g g g g t t t | g a g |
| t t t g g g g t t t | t a g t t t g t t t | t t a g g c g g t g | g g t |
| t t t g g g g g t t | t t t c g g t t g g | a g t t a t t t t c | g g g |
| g g t t t t t t t t | t t t t t t t t t t | t a t t t t t g c g | g t t |
| t t t g g g t t g a | g g a a a t t t t a | t a a t t t t a t t | t t t |
| t t t t t t t t a t | t a c g t g g t t t | t t t g t g g g g t | t g g |
| g g a a a g g g g t | g t g t t t c g g g | g a a a g g g t t t | a g t |
| a a a t t c g t t t | g a a t t t t g g g | t t t t t t t t t a | g t g |
| t t a t a t t a t t | t t t t t t t t a g | t g g g g t t g t t | t t t |
| t t c g t t t t t t | t t g t g a t t t g | a g t t t g t g t g | t t t |
| t t c g g t t t g c | g t t t t t t t t t | g t t t t t g g t t | t t t |
| a g a t t t a t t t | t t a g t t t t t t | t t t t t t a a a t | a t t |

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|                     |                     |                       |       |
|---------------------|---------------------|-----------------------|-------|
| t t g g g a g g t c | g a g g t t g t a g | a g a g t t g t a a   | t c g |
| g a g t t t t g g a | a g t t t g t t t t | a g a g t t a g t t   | a a g |
| g c g t t a g t t t | t t t t a t t t a t | a a a a t g g g g g   | t a a |
| t g a g a g a t t t | a a a t g a g g t g | g t g g a t t t g g   | a a g |
| a g g t g t t t g a | t t t t c g g t t t | t t t t t t g t g a   | a t g |
| t t g g g t t t t a | t t t t t t t t g a | c g t t g t t t t t   | t t t |
| g g g t a g g t a g | a g a c g t t g t t | g t a g g t t a a g   | g g c |
| a g t t t t a g t t | a g t t t g g a g a | t t t c g t g t t t   | t t t |
| t t a g g t t t t t | c g t t t a g g g t | t a t t t a t a t t   | a a g |
| g g c g g g g g a g | t t t t t g t t g a | g g t t t t t g t t   | t g t |
| t t g t t t g g g t | t t g a a t t t a a | g g t t g g g g a t   | t t a |
| g t t t a a t t t g | g t t t t t t t t a | g g g t g g a c g t   | t a t |
| a g t t t t a c g g | a t t t g g t g g a | g t a t t t t t a a g | a a g |
| t t t g t t t a t t | t g c g g t a g g t | t a g g g g t g g g   | t t t |
| t t t t t t a g a t | g t g a g t t t t t | g g g a t t t t t g   | a g t |
| a g t c g t a t t a | t g t t a c g a g g | g t g a a t g c g g   | t t g |
| a t a a g a a g t a | g g a g t t c g a g | g a t a t a g t t a   | a g g |
| a t g g t g g g g a | t c g g t a g g g t | t g g g g t a g t t   | g a g |
| c g g a t a t t t t | t t t t t t t t t g | t t t a t t t t t g   | t t t |
| a t g g a t g t t t | t t t t t g g g a g | t t g a t g t t t a   | t t t |
| a g t a g g a g g t | g a a g a a t t t g | t a t t a g c g t t   | t g g |
| a g a a t c g t t a | t a a g a a t a t t | t t t t t t t g t g   | a g t |
| a t c g t t t t t g | t t t t a g t t g t | t t t t t t t t a t   | t t t |
| g g g t t a t t t t | t t t a t a t t t t | t t a t a g a g t t   | t t t |
| t t a g a a g t g t | t t t t t t a t t a | t t a g t a g g t a   | g g t |
| a t t t t t t t a t | t t t t t t t a t a | t a g a t g a t t t   | t t t |
| g t t t t a t g g t | t t t t g a g a t t | a g a a t g g t t t   | g t t |
| g t g a g t t t t t | g g t t a a t t t a | g a t t a t t t c g   | t t t |

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agtcgagtga ttiigttaggg acgggaatagi aatattttcg ggttcgatta tattaatgtt 12300
a a t t a t a t t a   a g g t t a g t a g   t g t g g g t t a c   g t g
t t t g t t t g g t   g g g g g g a t t t   t a g a t t t a g a   g a t
t g t a t g g g t g   a g g g t g g t a g   t g g t t t a g g g   t t t
g g g g t g c g t t   t t t t t a c g t t   t g c g t t t a g a   a t t
a g a t t t a t a t   c g t t a g t t a g   g g t t g t t t g g   a g g
c g t g g t a g g a   g a a t a g t c g t   g t t a t c g t t a   t g a
t a g g g c g t t t   t t t t t t t t t c   g t a t t c g t t t   t c g
g a g t a g t t a g   a t g t t a g g g t   a g a a a g g g a t   t t t
a a a t t g a g g g   t t a g t g a t a a   a g t t t c g a t t   a t a
a t t t t t g g g t   t t t t t t g a g t   t t t a g a t t t a   g g t
t t g t t t t a t t   t g t t t g t a t t   t a g g t t t t t t   t t g
a g t t t g t t t t   t t a t t t t g t a   g g t t t t t t t t   a t a
g a t t t t a t t c   g t g a t a t a a a   t t g g g t t a a g   t t t
t t t t g g t t a t   t t t t g g g a t a   a a g t c g t a t t   t t a
t t t t t t t g a t   t c g t a t g t t t   t t t t t g a a g g   t t t
a g g t t t t t a g   t t t t t t t t t g   t a t a a g t t t a   t t t
t a t t t t t g t t   t g g t a g a t g t   t t c g t t t t t g   a a g
g a a t t t a g g t   t t t g t t t t t t   t t a g g a t t t a   g a g
t t t t t a g c g c   g g t g t t t t t t   t c g g t t a t t t   g t t
a t a g a t t g g g   t g t t a t t t g t   g t t t g t g a a g   t t g
g t t t g t t t t g   g t a a c g t t t g   t t g a a t g a t a   a a c
g t t t t a t t a t   t t g t t g g t g g   t t g a t t t g a g   a c g
a g t t t a t t c g   t t t a t t t a a t   a a a t g t t t g g   g t c
g g a t t t g g g a   t g g g t t a t a g   t g t t t t g t t t   t g t
t t a g a a t a a a   t g c g t t t t a t   a t t g g t t c g a   g g t
t t t t g t g a t t   a a t t g c g g g g   a g t a t g a t a t   a a t
t t t t t c g t t g   g a t a a t g t g a   g t g g t t t t t a   c g t
g g a t t t g t t t   t t t t t t t t g g   t c g g g t a g g g   t g a

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ggggtattga ttttaigtgtt tccgttttagg gagatttgat tccggagatt tggattattt 14040

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|--------------|--------------|---------------|-----|
| agtaatttgag  | ttgggttcgat  | tatgggggttt   | tta |
| tggattagat   | taatttagcgg  | taggaaagt     | tgt |
| gtaggtagagg  | atgataattt   | tgatggtagt    | agt |
| tgttatgagt   | tgttataagt   | aatataaacg    | tta |
| tttttcggttt  | ttttttgggtt  | ttttttatgggt  | ttt |
| ttagtttttat  | tttgggttttt  | tgttttgtggg   | tat |
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|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| c g a g g t a g t t | t t t t g t g g t t | t t t t c g g g g t | t a g |
| a g a t a t t a g g | t t t t a g g a t a | g t a g g g a g g a | g g g |
| a g g g g a g g g g | a t g g a g g g t a | g g t a g t g t t g | g t t |
| t a g g g t g g g a | g t c g g t t t a t | t t t t g t t t g g | g a a |
| t a t t a g t g g g | a t c g g a g t a g | t t t a g c g g g t | a t t |
| t t t g t a g g a t | a t t t t g t t g t | t g a g t g t a g t | a t t |
| t t t t t t t t t t | a t a t a g g t t a | t a g a a a t t t t | t t g |
| g a t t t t t t a t | t t t g t a g g g t | a t t a g g c g g t | g a g |
| a g g g t a g g g a | t c g g t t g a g g | t t t t g g g t g g | g g g |
| a g g g a t g g g g | a g t g t g g g g g | t t g g g g g t a a | g g g |
| g g g a a t t a g g | a a t g a g t g g t | t t a g g g t t t g | g a t |
| g g g t t g a g t t | a g g a a g t t a t | c g t g g a t a t t | t c g |
| t t t a t t g a g g | t t t c g g t g a a | a t t a t t t g g g | c g g |
| c g t g g g t a t t | a g g t a t t t t t | g t t t t t t g t t | t a t |
| t t t t g g g g g t | t t t c g g a g a g | g a a g g g g g c g | t a g |
| t a g g t a g a g a | g t t t a g g g a t | t a a g t t t t a g | a t g |
| a t t t t c g g t g | g t t t t a g t t t | t g g g g t t g t t | a t t |
| g g g g a t a g g a | a g a g g c g c g g | t c g a t t t t t t | g g g |
| a g t a t a g t a a | g g g a a g a g a a | g t a g a g t a g g | g t g |
| t t t t a t a c g t | t t g t a a t t a a | a g g t t t a g g a | g t a |
| g t a g t a a g g a | t a t a t g t g t g | t t t t g t a t a t | t t t |
| a a t t t a g a c g | g t t t t a c g g t | a t t t t t t t t t | t t t |
| t t g g t t t a c g | t t a a t a a t a a | t g a t g g c g g t | g a t |
| t g t c g g g c g t | t g t g t t a t a a | t g g c g t t t a t | a t g |
| t t t t a t g a a a | t a g g g g t t a t | t t t t a t t a t t | t t a |
| t t t a a g g t a t | t g t t t g a g t t | t a t t a g t g a g | t t a |
| t a g t t c g g t t | t t a a a g t t a g | t g t t t t g a a t | t t t |
| a a t a g a g a t t | a a g a g t t t t t | t t g t t t a a a g | g g g |

g g g t t g a g t t a g g a a g t t a t c g t g g a t a t t t c g  
t t t a t t g a g g t t t c g g t g a a a t t a t t t g g g c g g  
c g t g g g t a t t a g g t a t t t t t g t t t t t t g t t t a t  
t t t t g g g g g t t t t c g g a g a g g a a g g g g g c g t a g  
t a g g t a g a g a g t t t a g g g a t t a a g t t t t a g a t g  
a t t t t c g g t g g t t t t a g t t t t g g g g t t g t t a t t  
g g g g a t a g g a a g a g g c g c g g t c g a t t t t t t g g g  
a g t a t a g t a a g g g a a g a g a a g t a g a g t a g g g t g  
t t t t a t a c g t t t g t a a t t a a a g g t t t a g g a g t a  
g t a g t a a g g a t a t a t g t g t g t t t t g t a t a t t t t  
a a t t t a g a c g g t t t t t a c g g t a t t t t t t t t t t t  
t t g g t t t a c g t t a a t a a t a a t g a t g g c g g t g a t  
t g t c g g g c g t t g t g t t a t a a t g g c g t t t a t a t g  
t t t t a t g a a a t a g g g g t t a t t t t t a t t a t t t t a  
t t t a a g g t a t t g t t t g a g t t t a t t a g t g a g t t a  
t a g t t c g g t t t t a a a g t t a g t g t t t t g a a t t t t  
a a t a g a g a t t a a g a g t t t t t t t g t t t t a a a g g g g

|                     |                       |                       |                 |
|---------------------|-----------------------|-----------------------|-----------------|
| t a a g a t t t t g | t t a a t g t t t g   | t a a g a g a g a a   | g g g           |
| t t g t t t a t g t | t a t t g t g g t a   | g a g g g a t g g a   | t a a           |
| g g g t t t t t t t | t a a t t t t t t g t | t t t t a g g t t t   | t t g           |
| t t t a t t t t t t | g g g g g t t g g g   | g g a t a a g g t t   | t t t           |
| t g a t t t t a g a | g g t t t t a a a t   | a t t a a g g t a t   | a t g           |
| a a a t a t t a a t | a t c g t t a a a t   | t a a a a c g a g g   | a a a           |
| a t a t a t a t t g | g t g g t t t t t t   | t t a g g g a g g g   | a a a           |
| g a g a t a t t t g | g t t t t t t t a t a | t a t t t t t t t t g | a a t           |
| a t a a a a a t a a | a t a t g t t a a t   | t g g g a a t a a t   | t t t           |
| t t t t t t g t t t | t t a t t t g g a t   | g t t g t t t g t a   | t g g           |
| c g t a g t g g t t | t a c g t t t g t a   | a t t t t a g t a t   | t t t           |
| g t t a g g a g a t | c g a g a t t a t t   | t t g g t t a a t a   | t g g t t a t t |
| c g a g a t t a t t | t t g g t t a a t a   | t t g g t t a a t a   | t t g g t t a t |
| t t g g t t a t g g | t g g t g g g c g t   | t t g                 |                 |
| a t t t g a a t t t | a g g a a g t c g a   | g g t                 |                 |
| g g t a a t a t a g | t a a g a t t t t a   | t t t                 |                 |
| a a t a t t t a t g | a a t a t t a t g t   | a t g                 |                 |
| a a t a a g t t t a | a t t t t t t t a t   | g a t                 |                 |
| g t t t t t t t t t | a a a a a a g g t t   | t t t                 |                 |
| g g t t t t t t t t | t t t t a t t g g t   | g t t                 |                 |
| g t t t t g a t t a | t t t t t t t a g t   | t t g                 |                 |
| t a c g t t t a a c | g t t a a g g a g t   | a a g                 |                 |
| c g t t g g c g t t | t t c g t t t a t t   | t c g                 |                 |
| a a g t g t t t a a | t a a a t a a g t t   | c g t                 |                 |
| t t t g g t t t t a | t a t a t a t a g t   | t t g                 |                 |
| g t a t a t a t a t | t t t a t t a t t t   | a t g                 |                 |
| a c g t a t g g a a | t a t a a a t t t g   | a t t                 |                 |
| t t t a t t g t a t | t t t g g t t t t a   | a g a                 |                 |
| a a t t a a t t c g | g t a t t t a g a a   | g a a                 |                 |
| a g g a a t t t t t | t t g a a g g g t g   | t t a                 |                 |

|                       |                         |                       |       |
|-----------------------|-------------------------|-----------------------|-------|
| g t a t a g t t t t   | t g t t a t t t t t     | t g g t a g g a a a   | a t a |
| g t g t g g g g t t   | t g t a g a t t a t     | t t t t a t a t a t   | a c g |
| t g a t g t a g g a   | a t t a t t t t t t     | t t a t t t t t t t   | a t g |
| a a t t a t a a g a   | g t a a a t g t t t     | g a t a a t t t a g   | a a g |
| g a t a a a a g t a   | g a g a t t a a g g     | t t a g g c g t a g   | t g g |
| g g g g g t g a g g   | t t g g t g g a t t     | a t t t g a t a t t   | a a g |
| a a a t t t t g t t   | t t t a t a a a a a     | a t a t a a a a t t   | t a g |
| t t t a g g t a t t   | t g g g a g a t t g     | a g a t g g g a g g   | a t c |
| a g t g a g t t a t   | g g t t g t g t t a     | t t g t a a t t c g   | g t t |
| a a a a a t a a a a   | a t a t a g t t g g     | g c g t a g t g a t   | t t a |
| t c g a g g t g g g   | a g g a t t a t t t     | g a g g t t a g g a   | g t t |
| ttttattttt attagtttgt | aatttttagtt ttttagggagg | tgagggttgt agtgagtcga | 14100 |
| g a t t a t g t t a   | t c g t a t t t t a     | g t t t g g g t a a   | t a g |
| a a t t a a a a a t   | t a t a t a g a t t     | a a g a t t a a t g   | g t g |
| g t a a a t a a g g   | t a a a a t a a t t     | t g t a g a a a g t   | t t t |
| g t t t g a t a a a   | t g a g t t t a a t     | a a t a t t t t t t   | a a a |
| a g g g t t t t a g   | t g t t g t t a g t     | t t t t a t a t t a   | t a t |
| g t a g g t g t t a   | t t t a t t t a a t     | t t t a g a a a t a   | g a a |
| g t t t a a g g t t   | a t a a g t t a g t     | g a g t a g g a a g   | g t g |
| g t t t g g t g t t   | t t t t t t t t t a     | a t a a t t a t a t   | t g t |
| t g g g t a t t g t   | t a t a g t t t a t     | g a t g g a a g g t   | g g a |
| t g g g t t a g g g   | t a a a g a g g t g     | g t a c g t a t a g   | a g t |
| a a g t t a t g t g   | g t t g g a g t a a     | a g t t g t t t t g   | a g a |
| t t a a g g t t a g   | g t t g a g a a a t     | t a a g g a t g a a   | a t t |
| t t t g g t a g g a   | a a a t g a t t a t     | a g a c g g g a t t   | a g g |
| c g g t g g g g t c   | g a t a g t g a t a     | a g a t g t g t a t   | t g g |
| t a a g a a t t a g   | t a g g g t t a g g     | g a g t t a t t t a   | t t t |
| g t t g t t a t t t   | t t t t t t t t t t     | t t t t t t t t t t   | t t t |
| t a g g t t g g a g   | t g t a g t g a c g     | t a a t t t c g g t   | t t a |

|                                     |                                       |   |       |
|-------------------------------------|---------------------------------------|---|-------|
| t a a t t t t t t t                 | g t t t t a g t t t                   | t t t t a g t a g t                     | t g g |
| g t t t a a t t t t                 | t t t t t t g t a t                   | t t t t a g t t g a                     | g a t |
| t t t t g a a t t t                 | t t g a t t t c g t                   | g a t t t a t t t g                     | t t t |
| g t t t g a g t t a                 | t t a t g t t t a g                   | t c g g t t g t t a                     | t t t |
| t t t t t t t t t t                 | t t t t t t t t t t                   | t t t t t t t t c g                     | a t t |
| t t t t t t t t t t                 | t t t t t t t t t t                   | t t t t t t t t t t                     | t t t |
| t t t t t t t t t t                 | t t t t t t t t t t                   | t t t t t t t t t t                     | t t t |
| t t t t t t t g a t                 | a t a g a a t t t t                   | g t t t t a t t a t                     | t t a |
| t t a t t g t a g c                 | g t c g a t t t t t                   | t t g g t t t a g g                     | t g a |
| t g a g a t t a t a                 | g g t g t a t g t t                   | a t t a t a t t t a                     | g t t |
| t c g t a g t a g a                 | g a t g a g g t t t                   | t t t t t t a t g t                     | t g t |
| a a g t a t t t t t t a t t t t a g | t t t t t a a a t t g t t a g g a t t | a t t a t a t g t t t g g g t t a t t a | 15840 |
| t a t t t a g t a t                 | t t t t t t t t a a                   | t a g a g a t g g g                     | g t t |
| a t t t t t g a g t                 | t t a a g t a a t t                   | t t t t t t t t t t c                   | g g t |
| g c g t t a t t t t                 | a t t t c g t t a g                   | g t t t t t a t t t                     | t t a |
| t g t t g t g g t t                 | t t g t t t t c g t                   | t t t a t t a g g g                     | t t t |
| t g t t t g t t t t                 | t a t a t t a g g g                   | t t t t g t g t t t                     | a t t |
| t t t g a t t a t t                 | a c g t t a t a t g                   | g t t a g t t t t t                     | t t t |
| g t t a t t t t t t                 | c g g g a a g g t t                   | t t t t t g a t t t                     | t t t |
| g t t a t t t g t t                 | a g t a t a t g a a                   | g t t g g t t t t a t                   | t t t |
| g t t t t a t t t t                 | g t c g t t t a g g                   | t t g g t g t g t a                     | g t g |
| a t t t t t t a g g                 | t t t a a g t a a g                   | t t t t t t a t t t                     | t a g |
| t g t g t t a t t a                 | t a t t t a g t t a                   | a t t t t t t a t t                     | t t t |
| t t a g g t t g g t                 | t t t a a a t t t t                   | t g g g t t t a a g                     | t a a |
| t g g g a t t a t a                 | g g t g t g a a t t                   | a t t a t a t t c g                     | g t t |
| t t t g a g a a t t                 | t g t a a t g a t t                   | t a a t t t a t t g                     | g g t |
| g g a a t g t a a g                 | t t t c g t g a g a                   | g t a g a g a t t a                     | t g t |
| t a t t t t a a a t                 | a g t a t t t g g t                   | g t a t a a t a g a                     | t g t |
| g t a g g g g a a a                 | g g g a a g t g a a                   | a g g a a a t a a a                     | g a a |

|              |              |               |           |
|--------------|--------------|---------------|-----------|
| gtgcggagaa   | gttggttgga   | cgtgggaggg    | ttt       |
| tgagggtgttg  | tgagttattt   | aggggggatatag | gtt       |
| agagggttgga  | agtagaaatg   | taatggagag    | aat       |
| ttaaagaggg   | ggtagaggag   | tatgagtgag    | tcg       |
| aagttttatag  | ggatcggaaa   | taggcgtagg    | gta       |
| cgtcggagtg   | agtatttaata  | aagtttttttt   | ttt       |
| tcgatgggtgt  | tcgggatttta  | tttacgggat    | agt       |
| ggcggagggt   | cgtaggggggc  | gggggttgagt   | tag       |
| ttagggtgagg  | aagaatcgtt   | tagtaatttag   | tta       |
| taatggtatt   | gtagggataa   | atttttggtatt  | aat       |
| gggggttggtt  | cgagatcgtt   | tatagcgggtt   | agt       |
| agtaggttcg   | tttgttggtt   | taggttcgtt    | tttttcggg |
| agaggaggag   | ttatttggag   | 17580         |           |
| tttttattttgg | agttttaagtt  | gatagagcgg    | cgg       |
| agtaatttttg  | gttgagggaag  | agtaatttaaat  | ggg       |
| agggttttaag  | gaaaaatttttt | aatagtttttag  | ttt       |
| ggttttatttta | tttaggggcgt  | tgaggggatttt  | att       |
| ggggaggaggt  | tttaggtagg   | gttaggttttt   | ggg       |
| gggaggaggtt  | gttttaggggg  | atgggggttttg  | gaa       |
| tggggggttgt  | taggtttcgta  | agagatttgat   | att       |
| ttgttttgga   | tatttaagggtt | tttttttttttt  | tgg       |
| aggaggcgggt  | tacgttgttta  | ttagtagtag    | gtt       |
| gaaatagcgt   | tttttagtttg  | tttgatagggt   | tgg       |
| atttgattaa   | gcgagtaaat   | ttaaggatat    | ttg       |
| ttttaaggat   | tagcgtgttt   | aagatatatttg  | gag       |
| ttttttattgg  | ttgggtagtt   | gtttgtttaa    | tag       |
| gttttggaatga | tgatatatttg  | tttatatttg    | gta       |

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<211> 19

<212> DNA

<213> A r t i f i c i a l   S e q u e n c e

<220>

<223> D e s c r i p t i o n   o f   A r t i f i c i a l   S e q u  
S y n t h e s i z e d   P r i m e r   S e q u e n c e

<400> 7

c a g g c c a g t g   g a g t g g c a g

<210> 8

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequ  
Synthesized Primer Sequence

<400> 8

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<210> 9

<211> 126

<212> DNA

<213> Homo sapiens

<400> 9

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<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequ  
Synthesized Primer Sequence

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<210> 11

<211> 21

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Synthesized Primer Sequence

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<213> Homo sapiens

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<211> 23

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<220>

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Synthesized Primer Sequence

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<223> Description of Artificial Sequ  
Synthesized Primer Sequence

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Synthesized Primer Sequence

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<211> 20

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequ  
Synthesized Primer Sequence

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequ  
Synthesized Primer Sequence

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<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequ  
Synthesized Primer Sequence

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<210> 19

<211> 159

<212> DNA

<213> Homo sapiens

<400> 19

g a a c g c c a t t   a t a g c a c a g c   g c c c g g c a t c   c a g  
c g c c a t c a t t   g t t a t t a g c g   t g g g c c a g g g   a g g  
g g a g a g a t g c   c g t g g g a c c g   t c t g g g t t c g   c a t

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## DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] It is a base sequence figure showing the base sequence of the sense strand of the SHP1 gene genomic DNA used with the hematopoietic organ tumor cell detection method concerning this invention.

[Drawing 2] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#).

[Drawing 3] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) and [drawing 2](#).

[Drawing 4] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 3](#).

[Drawing 5] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 4](#).

[Drawing 6] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 5](#).

[Drawing 7] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 6](#).

[Drawing 8] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 7](#).

[Drawing 9] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 8](#).

[Drawing 10] It is an arrangement figure showing a continuation of the base sequence of the sense strand in the genomic DNA of SHP1 gene shown in [drawing 1](#) - [drawing 9](#).

[Drawing 11] It is a base sequence figure showing the base sequence of the antisense strand in the genomic DNA of SHP1 gene used with the hematopoietic organ tumor cell detection method concerning this invention.

[Drawing 12] It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#).

[Drawing 13] It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) and [drawing 12](#).

[Drawing 14] It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 13](#).

[Drawing 15] It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 14](#).

[Drawing 16] It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 15](#).

[Drawing 17] It is an arrangement figure showing a continuation of the base sequence of the

antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 16](#).

[[Drawing 18](#)]It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 17](#).

[[Drawing 19](#)]It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 18](#).

[[Drawing 20](#)]It is an arrangement figure showing a continuation of the base sequence of the antisense strand in the genomic DNA of SHP1 gene shown in [drawing 11](#) - [drawing 19](#).

[[Drawing 21](#)]It is a base sequence figure showing the base sequence of cDNA of SHP1 gene used with the hematopoietic organ tumor cell detection method concerning this invention.

[[Drawing 22](#)]It is a mimetic diagram showing the outline structure of SHP1 protein used with the hematopoietic organ tumor cell detection method concerning this invention.

[[Drawing 23](#)]It is an amino acid sequence figure showing the amino acid sequence of SHP1 protein shown in [drawing 22](#).

[[Drawing 24](#)]In the genomic DNA (sense strand) of SHP1 gene shown in [drawing 1](#), it is a base sequence figure showing the part of CG arrangement methylated on a CpG island.

[[Drawing 25](#)]It is a chemical reaction explanatory view showing the process in which cytosine is changed into uracil in the heavy sulfite treating used with the hematopoietic organ tumor cell detection method concerning this invention.

[[Drawing 26](#)]It is a mimetic diagram showing the state where cytosine is changed into uracil and the methylated cytosine is not changed by the heavy sulfite treating used with the hematopoietic organ tumor cell detection method concerning this invention.

[[Drawing 27](#)]It is a base sequence figure showing the base sequence after carrying out bisulfite processing to the sense strand of the SHP1 gene genomic DNA used with the hematopoietic organ tumor cell detection method concerning this invention.

[[Drawing 28](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#).

[[Drawing 29](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) and [28](#).

[[Drawing 30](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) - [drawing 29](#).

[[Drawing 31](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) - [drawing 30](#).

[[Drawing 32](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) - [drawing 31](#).

[[Drawing 33](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) - [drawing 32](#).

[[Drawing 34](#)]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in [drawing 27](#) - [drawing 33](#).

[[Drawing 35](#)]It is an arrangement figure showing a continuation of the base sequence after

carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in drawing 27 - drawing 34.

[Drawing 36]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the sense strand in the genomic DNA of SHP1 gene shown in drawing 27 - drawing 35.

[Drawing 37]It is a base sequence figure showing the base sequence after carrying out bisulfite processing to the antisense strand of the SHP1 gene genomic DNA used with the hematopoietic organ tumor cell detection method concerning this invention.

[Drawing 38]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37.

[Drawing 39]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 and drawing 38.

[Drawing 40]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 39.

[Drawing 41]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 40.

[Drawing 42]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 41.

[Drawing 43]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 42.

[Drawing 44]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 43.

[Drawing 45]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 44.

[Drawing 46]It is an arrangement figure showing a continuation of the base sequence after carrying out bisulfite processing to the antisense strand in the genomic DNA of SHP1 gene shown in drawing 37 - drawing 45.

[Drawing 47](a) - (d) is a mimetic diagram showing the step of methylation specific PCR used by this invention, respectively.

[Drawing 48](a) It is a base sequence figure showing the primer for PCR used in Example 1 whose - (b) is an example of operation of this invention, and (c) is a base sequence figure showing the base sequence of SHP1 gene (genomic DNA and sense strand) which the primer for PCR used by (a) - (b) recognizes.

[Drawing 49](a) It is a base sequence figure showing the primer for PCR used in Example 2 whose - (b) is an example of operation of this invention, and (c) is a base sequence figure showing the base sequence of SHP1 gene (genomic DNA and sense strand) which the primer for PCR used by (a) - (b) recognizes.

[Drawing 50](a) It is a base sequence figure showing the primer for RT-PCR used in Example 3

whose - (b) is an example of operation of this invention.

[Drawing 51](a) It is a base sequence figure showing the primer for real time RT-PCR used in Example 4 whose - (b) is an example of operation of this invention.

[Drawing 52](a) Are the primer for methylation specific PCR used in Example 5 whose - (b) is an example of operation of this invention a shown base sequence figure, and (c), (a) It is a base sequence figure showing the base sequence of SHP1 gene (genomic DNA and sense strand) which the primer for methylation specific PCR used by - (b) recognizes.

[Drawing 53](a) is a figure showing fluorescence in situ hybridization (FISH), and (b) is a figure showing one typical data of the analysis result of heterozygosity loss of SHP1 gene in an ALL patient.

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## DRAWINGS

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### [Drawing 1]

```
ccctctagtt gtgcacgtg tagcaaat gtctctctcc aacacacacg ggcacagggc      60
ctgcatgcat cctgttttgc aacacagtcg ccagccagtg gggagagagt tcatgttag      120
actacacccc cctccagtg tttagggac gctgtctctt agggagaggg ttgaccttc      180
actccctctt cccagtgctc ttatgtttgc tctgttgctc aagtcctacg aagccacgga      240
tcctgagatc ggcagcgtgt caggaagcat gaggcgctg ttctgcccgc ccgtgacct      300
gacaccccat ggcctgctg ctgttgccag cgtggccgac tcctgagagt tggccctcgc      360
ttgtgcactc gacaggggag gaaagccctt gatgttcacg acattatcac atgagcctgt      420
gacttagcct tgggtcagt ctctgggagc cctgacacac cactctctct ctccctgct      480
tcctctgccc ttctcagggc cactccacct gacagctcac tcctctatgt cctggctggg      540
cctaaccttg cccaggggac taacccctac tgaggctcct ccccttccac ccggagcagt      600
tgagagcttg gctgagctcc ctccagccgc tgggtggctg gctctgacac ggggtacat      660
ccctctctga ggaactgggc tgttaggagt ttctcttagg ccttttgatt taccctcag      720
gagaggtttc ccccttggtg tgccttctct cagccagagt taactctcgg tctgttccc      780
taacacatcc cccgcgcgta tctcagcttg agctccaggt ggcgtccagc gtgctctctc      840
ctctcccgag ggaagcgacc cctggaccag caggccggcc tgcgtactcc ccgttttgg      900
gctgcagggc agctggcgc tctggcaggt ctggggcgc cccgcgcgcac cctgtccttt      960
tcctggagac tattgttcca ggttttgcac ctgacgtcac attgacctgg caggcagcat      1020
cagagcagaa ctgacctgatt actgacaggt tcttctctac ctgacctgag ccactgtcca      1080
cagctgtccc cctgacctga cccgcgcgcc tccgaccttc cagctgtcct tccctctccc      1140
TACAGAGAGA TGTGTCCCG TGGtagtc ccgggcacac tggggtccc agtctctgt      1200
tagttttgga gggagggagg gctttgtga tgcctacccc gactgtgtg aagctgagt      1260
cgatctgccc ctgcccagag cctgtttcag gtcctatgca acttcccttt ccggagagt      1320
gtgagacccc cggctcact cagctctctc tgcacctctt ttacatttt cccctgaca      1380
agtgtgata tgtctctccc attgctttc taacttcagc ctctgggctc ctgcttctgc      1440
ctctctgctt ggaactgtcc cctgggttag ctacacacac ctcaacata gcatcagag      1500
gacacccgag aagccctccc cagctccagc caattctccc gacttccccc cactcagat      1560
ttgttccact ctcttttgtt tcccttcaat tcccttccc ctgactcatt caattcagag      1620
gtcagtggtg agctctatt atgacacagg tctgttttaa gatgtgtga atactggagt      1680
gacacagaca gactgtct ctgtctccc gggcttata ttccagtgga aggttaccag      1740
ccgacacact aacacatca attgtctat tgcagttct ccaggtatt ccgacagaaa      1800
tagacagcct tggccgggtg tagtggttca cactgtgtat ccagcactg tggagagctg      1860
aggcagaggg attgtttgag cccagagatt tgagacagc ctggccata tagtagaac      1920
ctgtctctac aaaaataag caattagctg gctgtgtgga caccctctct gtgttccag      1980
```

### [Drawing 2]

|  |      |
|--|------|
| ctatgagag gataagiga gaggutigt ttagootgag aggtcaaggo tgcagtoago | 2040 |
| gatatitga oomotigooa oagootggg oguonagtg agootigtg toaanaama   | 2100 |
| aaaaaanaa gaaatgaao oagotbaa tgotagaaag tgaotggatg tgcagtgao   | 2160 |
| attatagut gaggagtao gaggagoot oongaggag tgaottiga gogagaooo    | 2220 |
| gagtagagag gaagagago tggootigt agtagtgt oagagtaaa gatatotag    | 2280 |
| gagagagaa tagtagaoa aaagootaa tggagaaaa aaaaaaaa ggaagtgig     | 2340 |
| oogtagag aggaootag tggagagag gaaagagaa agagattag aocagttag     | 2400 |
| agtagagtg tgaagtgaa aaocotgaag agtagagtg agacagctot gtagtoag   | 2460 |
| oactitggg ggcagaggg ggaagtgig ttagagtag gattitaaa oagootggg    | 2520 |
| caacatagag agacocata tatattaaa aaataactg gtagtagtg ooaagcagtg  | 2580 |
| tgttagtoot agcagtttg gaggtagag tggagagtg aottagagc aagagttca   | 2640 |
| gaacacootg agacacatag gtagagagc ootototat agacacagc oaatattaa  | 2700 |
| taataaana tagotggat tagtgagtg oacotgtgt otagttat tggagagtg     | 2760 |
| aggaagagag aocacotag oaaagagtg agagtagaa gtagtagtg ttagtaant   | 2820 |
| gaactioag otaggtgta aggaagatt otgigtaaa aaaaaaaa aaagagagag    | 2880 |
| gaggaagag aggaagagaa ggaagagaa aaagagaaa gaaagagaaa aggaagag   | 2940 |
| ogagaagaa gaagagaa gaaagagaa gaagagaaa aggaagaaa gaagagaaa     | 3000 |
| agtagagc oagtagag aggaagag aggaagaaa aagtagaaa oagtagaaa       | 3060 |
| gaagagaaa aagtagaaa oagtagag otgtagtg aagtagtaa toocagagc      | 3120 |
| ttagagagag gaggagagtg gtagagag tagagagtg agacagagc tggagagag   | 3180 |
| gtagagagc ttagtagc aagtagaaa aaaaaaat agtagagc agtagagag       | 3240 |
| oactgtag oacagtag agtagagtg aggaagag atagtagaa oacagagag       | 3300 |
| gagagtgaa gtagagag agtagtag tgaotagag otagtagaa gtagagag       | 3360 |
| oacotagaa aaaaaaaa aaagagaaa gaagagtg oacotgatt aagtagat       | 3420 |
| gtagagtg tagtagtg gtagagag ootgagtg otagtagtg oacotagtg        | 3480 |
| otgagagag oacagtag atotgtgaa ttagagagc aagtagag ttagtagag      | 3540 |
| gtottagtg oacagtag gtagtagtg attagtag oagtagag gtagagag        | 3600 |
| oacotgtg agtagtag otagtagtg oagtagag ttagtagag otagtagag       | 3660 |
| agtagagag otagtagag aottagtag oacagtag gtagtagag gtagagag      | 3720 |
| tgaotagtg atotgtg gtagagc ttagtagag oacagtag otagtagag         | 3780 |
| gaotagtg otagtagag otagtagag ttagtagag oacotagag otagtagag     | 3840 |
| oacotagag ttagtagag ttagtagag aagtagag otagtagag gtagtagag     | 3900 |
| oacotagag oacotagag otagtagag ttagtagag gtagtagag ttagtagag    | 3960 |

### [Drawing 3]

|   |      |
|---|------|
| ttagtagag ttagtagag ttagtagag ttagtagag ttagtagag           | 4020 |
| gtagtagag agtagtag aaagtagag agtagtag ootgagag ttagtagag    | 4080 |
| taagtagag atttagagaa tagtagag otagtagag agtagtag ttagtagag  | 4140 |
| agtagtag otagtagag ttagtagag ttagtagag ttagtagag agtagtag   | 4200 |
| ttagtagag ttagtagag ttagtagag ttagtagag ttagtagag ttagtagag | 4260 |
| gtagtagag ttagtagag agtagtag gtagtagag gtagtagag otagtagag  | 4320 |
| taagtagag gtagtagag gtagtagag tagtagag agtagtag ttagtagag   | 4380 |
| agtagtag gtagtagag otagtagag ttagtagag ttagtagag aagtagag   | 4440 |
| gtagtagag gtagtagag gtagtagag gtagtagag ttagtagag agtagtag  | 4500 |
| ttagtagag agtagtag aagtagag aagtagag otagtagag ttagtagag    | 4560 |
| oagtagag otagtagag otagtagag otagtagag ttagtagag gtagtagag  | 4620 |
| oacotagag gtagtagag gtagtagag gtagtagag gtagtagag gtagtagag | 4680 |
| otgtagag aagtagag aagtagag otagtagag otagtagag ttagtagag    | 4740 |
| oagtagag gtagtagag otagtagag ttagtagag ttagtagag otagtagag  | 4800 |
| aatagtag ttagtagag ttagtagag ttagtagag aagtagag otagtagag   | 4860 |
| ttagtagag otagtagag gtagtagag gtagtagag otagtagag ttagtagag | 4920 |
| taagtagag ttagtagag gtagtagag gtagtagag otagtagag aagtagag  | 4980 |
| agtagtag ttagtagag gtagtagag gtagtagag gtagtagag ttagtagag  | 5040 |
| gtagtagag ttagtagag ttagtagag ttagtagag ttagtagag aagtagag  | 5100 |
| gtagtagag ttagtagag gtagtagag aagtagag otagtagag gtagtagag  | 5160 |
| ttagtagag ttagtagag otagtagag ttagtagag gtagtagag ttagtagag | 5220 |
| oagtagag ttagtagag ttagtagag gtagtagag gtagtagag otagtagag  | 5280 |
| gtagtagag aagtagag ttagtagag gtagtagag ttagtagag otagtagag  | 5340 |
| oagtagag gtagtagag ttagtagag aagtagag ttagtagag otagtagag   | 5400 |
| gtagtagag gtagtagag ttagtagag aagtagag ttagtagag otagtagag  | 5460 |
| ttagtagag otagtagag gtagtagag aagtagag otagtagag gtagtagag  | 5520 |
| oagtagag ttagtagag ttagtagag otagtagag otagtagag otagtagag  | 5580 |
| gtagtagag otagtagag gtagtagag aagtagag aagtagag otagtagag   | 5640 |
| agtagtag gtagtagag gtagtagag aagtagag aagtagag aagtagag     | 5700 |
| agtagtag gtagtagag ttagtagag aagtagag gtagtagag ttagtagag   | 5760 |
| aatagtag ttagtagag aagtagag ttagtagag aagtagag otagtagag    | 5820 |
| oagtagag aagtagag ttagtagag ttagtagag aagtagag otagtagag    | 5880 |
| tttagtag aagtagag otagtagag ttagtagag gtagtagag gtagtagag   | 5940 |



# [Drawing 4]

|   |      |
|---|------|
| agctcgctgc aacctcggtt tccctggctt cagtgattct cctgctttaa cctcccgagt | 6000 |
| agctgggatt acaggcgccc accacatgc ccagctattt ttttgtatt tttagtagag   | 6060 |
| agggggttto aocagtgtg ccaggtatgt ctgctctctc tgaactgtg atcggcagc    | 6120 |
| ctgggctcct caaagtgtg gattacagg cgtgagccac tggcgccggc caacttccg    | 6180 |
| ttttatacaa cccatgcaaa cagatcccg ctgagacaaa agagccttcc ctgtacocct  | 6240 |
| aaagtctccc agaatgtgt cccagttagc attttttt ttataaagt atgctgtcc      | 6300 |
| octctatata ccttaaaaa gttatgtaga gaaacagtg tctcccccag cctgtctct    | 6360 |
| cagccaccca gtttccctcc ctaggggag ccacacatat gtttttcta tctatccct    | 6420 |
| gttgagctgc ttttccctgt ttgtgttg ccgtgtgat gttgtattt ggaattacg      | 6480 |
| gtaggcagca tcatatacct tagtgttag gacctctag atcacccag cctgagaaaa    | 6540 |
| tcagccatgg tggagcctt gtcccccag ccccccagga tagcccccct gttggatg     | 6600 |
| ctgggctagg gacaggcct agggacaga attagaaag acccagtgt acaggctgc      | 6660 |
| tcaggctcat gttgtccat cctctgcac agtggcgtg acaaacctga tatgtgtt      | 6720 |
| agaggaggcc acccttctct ctgtcaagca ttggcaagt cttaactatt agtctctgc   | 6780 |
| tcacatggca gccccttgg acaaggagga tcttaactct tcttcttga agccctgagg   | 6840 |
| gctgtgtat aggagttcaa agaatggtt ttggaacgg actgtctgg ttgaactct      | 6900 |
| ggccctgag ctgactcact gctggactca ggaatgct taactcctt gacccctag      | 6960 |
| ttactgtct gttaactgt aagatagcc cctgttcat aggtctgag tgaacacca       | 7020 |
| atcagacag gcatgtgac gccattatag caccagccc ggcctccag aggaactact     | 7080 |
| cgtgacagt tctcccccgc atcatgtta ttacgtgg ccaggaggg ctgctataa       | 7140 |
| gcagctggt gaggaggag agtggcgtg ggaacgtct gtttgcctg cgtgagtat       | 7200 |
| tatctggcc tggagtgtg aaggacaca tctgtctta ctgactgtt tctccatat       | 7260 |
| gtgaactgc atgctctga gcttggatt gaaagctgt ggaagtgag cccctccac       | 7320 |
| acccccagt ccaactgct ctgtctctt tccctgtg tctctataa cgaagatga        | 7380 |
| aagtgaatt ccccaaggc tggcccgcc ctctctctt ccccgccct cggctgccc       | 7440 |
| caggcaatg cagtggcag cccagaaatg ggaacaccc ggtgtgtgag cccgcccggc    | 7500 |
| actggaagct cactctgag cttaactcct gacgtctct cctgcccaga ctactgac     | 7560 |
| ctctctatc cctgcccgc ctctctctgc ggaagccgc agatgctga gtaaggcc       | 7620 |
| tgcacccac gtagaagg aggaagggt gctgtgtgc caaggaacc cctctaatg        | 7680 |
| cctgctgg gcccacag tcttttcac gacagctcag tggctgcat gacagaaac        | 7740 |
| tctctaaagg ccgagctgt cactgtatgt tctgctctg cccactcgc aagaaccag     | 7800 |
| gtgactctg cctctcctg agtaggtg gcccaccca aacccagga tttgtcac         | 7860 |
| tctctgtg catccaggcc ctgaacact ccttctgtt tcccgctg agtctgact        | 7920 |

# [Drawing 5]

oocngtgtg toocgtgoc ocaocococa caotococat coctgtgtgt gccoacocat 7980  
 gcooctgtgt gcoocococo aggaococag coctococat coctococat totactocgt 8040  
 oocngtgtg ootocococo tgggtgocgt oagggg9999 GATCAGGTGA CCAATATTGA 8100  
 GATCAGAGAC TCAGGGGATT TCTATGACT GTATGAGAGG GAGAAATTTG CCACTGTGAC 8160  
 AGAGCTGTGT GACTACTACA CTCAGACACA GAGTGTGTGT CAGGACCGGG ACAGGACCAT 8220  
 CATCAGACTC AAGTACCGCG TGAAGTGTGT CCAATGCACT AATGAGAGgt gagggtocg 8280  
 caccococgc attcocaagc aggggtgagc oaggtococo ootgacagc caggagagca 8340  
 agggagctg cagocggcgc tggctacoc ccatcococt coctococat acbagctgg 8400  
 gctocaggt tootocotoc ctgtgtgtgt aggaocgtgt gctocagagc ctacocacac 8460  
 aoccttoca cctacococg aggaagcagc agaaagctgc ctocococat tocgagagc 8520  
 ctggcgcgt caccocaggt coctocggag acagggagc cactgtgtgt ggcagagctg 8580  
 toctgagagc cagctgtgt gtagagagc tottctctct ctggagagc gctgtcctc 8640  
 otagtctgc coctocococ agaaagctgt aggaagctgc gtagagagc ctggagagc 8700  
 gtagagagc aggtgtgtgc aggaagctgt aggtgtgtgc coagocococ ctggagagc 8760  
 ggtgtgtgt gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 8820  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 8880  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 8940  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9000  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9060  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9120  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9180  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9240  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9300  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9360  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9420  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9480  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9540  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9600  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9660  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9720  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9780  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9840  
 gtagagagc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc aggtgtgtgc 9900

[Drawing 6]

totggggg toctgggg agtgcococ aggggggg gagggtgtt agggagagc 9960  
 ggtgcococ toctgcococ coctococat gtagagagc aggggggg toctgcococ 10020  
 otagagagc gtagagagc coctococat toctgcococ toctgcococ gagggtgtt 10080  
 ttttttcat coctgcococ otagagagc gtagagagc gtagagagc ttttttcat 10140  
 aggggggg gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10200  
 aggtgtgtt gtagagagc otagagagc gtagagagc gtagagagc gtagagagc 10260  
 coctgcococ otagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10320  
 toctgcococ gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10380  
 coctgcococ gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10440  
 aggtgtgtt gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10500  
 coctgcococ gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10560  
 otagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10620  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10680  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10740  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10800  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10860  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10920  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 10980  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11040  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11100  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11160  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11220  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11280  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11340  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11400  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11460  
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 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11760  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11820  
 gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc gtagagagc 11880

# [Drawing 7]

|            |           |           |           |           |            |       |
|------------|-----------|-----------|-----------|-----------|------------|-------|
| acagccctg  | ccacagctg | ctccctcat | ctacacgtg | tcacccctc | acgacagag  | 11940 |
| ggcccatct  | ccacacccc | ccacagagc | tcacccctt | ccacagagc | tcacccctt  | 12000 |
| ccagcagtg  | ctcccccac | ccacagagc | ggtgcccc  | tgctccac  | ctcctgtg   | 12060 |
| acccctcc   | tcacccata | cagctgctc | ccacccctg | ctgcccac  | tcacccgac  | 12120 |
| gcctctgg   | ttctgagcc | agctgctc  | gttagctag | gaggtctg  | ccagctgtg  | 12180 |
| gtgctcct   | ggtacccc  | gacatctg  | ctcctctc  | gcacccctc | agttagccac | 12240 |
| AGCCGAGTA  | TGCTGAGG  | AGCGACAGT | AACATCCCG | GGTCGAGTA | GATGAATGC  | 12300 |
| AACTACATCA | AGTgagag  | gtgacccc  | gtgacagag | agctgagc  | ctggaatc   | 12360 |
| ccgtctgt   | gggggccc  | tgatccag  | gacagctg  | ccagccgac | gtgctctt   | 12420 |
| tgctgctg   | agctgagc  | gtgctgag  | ctgtgctg  | gcacaggg  | tcactgtt   | 12480 |
| ggctgctg   | tcacccct  | tgctgagc  | AGCAGCTCT | AGCAGCTCT | GAGAGCCTA  | 12540 |
| AGAGCTAGTA | CCGAGCCAG | GGTTGCTCT | AGCAGAGCT | CAATGACTT | TGCGAGTGG  | 12600 |
| CGTGGAGGA  | GAACAGCGT | GTGATGCTA | TGACAGCCG | AGAGGTGAG | AAAGCCCGG  | 12660 |
| tgagccccc  | cccttccc  | gcctccgc  | ccgtctgt  | gtctgcca  | tgagtgag   | 12720 |
| gagcagct   | ctgacagc  | agagagct  | ctgacagc  | agctgagc  | ccgtgtgg   | 12780 |
| aacctgag   | ctagtcac  | gtctgact  | acacagct  | cccccagct | ccctgctg   | 12840 |
| ctcctgag   | tccttgag  | tcacccac  | gttccagc  | gtctctct  | ctcctaccc  | 12900 |
| ctgcccac   | gtctgact  | cagccccc  | ctgtctcc  | tgcccatag | ctctctgtg  | 12960 |
| agctgccc   | tgacctga  | gctccccc  | ccacagcc  | ctctgtgt  | ccctgagct  | 13020 |
| gctccccc   | gtgacccc  | ctgctgag  | ttcctctt  | tgagactt  | ctcctgag   | 13080 |
| tcctgctc   | cttgggga  | agctgccc  | ctgacagc  | gcttccag  | ctgctgct   | 13140 |
| tcctctgac  | ccgacgct  | ctctgagc  | ctgacccc  | ccgacccc  | cagctctt   | 13200 |
| agctccac   | cccttctt  | ccacgctc  | ttctgtga  | gagctgct  | ctctccac   | 13260 |
| tcctctgac  | tgagagag  | ctgcttct  | agacacag  | ccgacgct  | ctctctgt   | 13320 |
| gagctccg   | ctgcttct  | ccagccct  | gagctgct  | tcctctt   | ccgacacg   | 13380 |
| tcctccag   | gtgtctcc  | ccgtccct  | gtctgtga  | gctctgag  | gacacagc   | 13440 |
| ccagctgag  | gtgtctct  | gtctgtga  | ctgtgtgt  | tgacagct  | ccgacacct  | 13500 |
| gctgctg    | gcacgttg  | ttgctgac  | acagctga  | ccgtgagc  | gtgtgctg   | 13560 |
| gctccccc   | ctgtgtgt  | tgctgtga  | ccgacagc  | agctctgt  | ctctctgac  | 13620 |
| agccatcc   | tcctccac  | aacgttgt  | gcctgccc  | gacacagc  | acatagac   | 13680 |
| gacctgga   | tgagccag  | tgccctgt  | gtgtctct  | ccacacag  | ccctccct   | 13740 |
| ccagAACAA  | TGCTCCAT  | ACTGCCCGA | GGTGGCATG | CAGCCTGCT | ATGGGCCCTA | 13800 |
| CTCTGTACC  | AACGCGGG  | AGCATGAGC | AACGGAATC | AACTCCCTA | CCTTAGAGT  | 13860 |

# [Drawing 8]

CTCCCGCTB BACAATgtga gtggccccc egccctggcc ccttcggga gtccctccct 13820  
 ggcctgttcc tccctctga tgggttagg tgggttagt gagggtgtcc gagagagag 13880  
 gggccctga cccctgtcc tgggttagg GAGAGCTGAT TGGGAGATC TGGCATTACC 14040  
 AGTAGCTGAB CTGGCCGAC CATGGGCTGC CAGTAAGCC TGGGGGTGC CTCAGGTTGC 14100  
 TGGACAGAT CAACGAGCG CAGGAAAGT TGGCTCAGC AGGGCCGATC ATCGTAGCT 14160  
 GCAGgtgagg gtgtatcc tgtgttagt agtgacagc gaggagtaa tactgtatg 14220  
 tgccttggc tgttatagg actataacg ttatgtgca cattgtgtc cctccgtca 14280  
 ccccccgtt cctccgttc ccccttggc tccagaccc tgggtgato gtggtggaa 14340  
 ccagccccc ttggccctc tgccttggg tatcttccc agagccctc cgggtgtac 14400  
 actctggcc aacccgtca actacaggg agggacccg gacccgttg ctggccagg 14460  
 ccagctagt caggccagg ccggccagg accccagta ggcctgttc cggctgtc 14520  
 cgtttctct cgggttccc tctgttgg ttcttctcc aggaactct atgagcgtg 14580  
 tgcctccat tccctcttt ttccctcgg tggcccccg acttcgctt cctctgct 14640  
 ctgctctc tccagcttc cccagcagt gcccctctt ggccccccg gctgttgg 14700  
 gttgtgtgt gcttcttgg ggcctccat aactctgt totcttccc cgtgttgg 14760  
 tgcctaggg cccctgtga aggtcagg gtgggtgt caggaggg cagggttcc 14820  
 ccaggtgag cccctgtgt ggcctgctt ggtgtatga gaggcgtg gtccaggcc 14880  
 cctccgtta cccctgtgt ggtgtgga aactctta actttctaa cccctgtt 14940  
 cctctgtt aactcagg totcgggt gtgtggaa cctcaggc cctctgtt 15000  
 gtgtctgga tctgttga cctccccc tgggtgtgt tactgtta gtaactca 15060  
 tccagcagg actagggaa tgggacccg ccttgcacc gtaactccc actccctgg 15120  
 tggcccccg gctgtggcg gctgtgtgt tccctcttt ccccccag ctgttctc 15180  
 tggcccccg cagctgtgt aactataga gtcccccga tgggtgtcc gggagcgg 15240  
 cctgtctgt tgccttcca gggccagg cctcccccg agtaactcc ctgtccccc 15300  
 ggcacacaa cctccccc cctctgga gcccctggg ctttcttgg agttacag 15360  
 agggagtg gtgtgggg ttattttga cactctgtt ttgaattg acagcggc 15420  
 tccagctcc agctgtgt gctcagtg ggtgtgtgt ggtgtgtgt tgggtgtgt 15480  
 ttggccccc ctgggagg agagacctt cctctgga tgcctccgt agtccctga 15540  
 cctgtccc ccccccgt ctgtctgg gctctctt cctcagtg aggtttga 15600  
 gggagagg agtgagga tgcctaga cctccctaa cctctcag gaggaggg 15660  
 gggagagg gttacctgg agcccccg ccaggtgtg gtttgggg tccaggtct 15720  
 tccaggtgg gggagccc tccctagg tggagtg gggagagg tggagagg 15780  
 aggtgtgt gggagggg agccagtg agcccccg agccagggt gggaggtg 15840

# [Drawing 9]

catgacagc tggccccc tccctctca cttgcccgt ggcctgggc aactctccc 15900  
 cctccctgg aggtcagg tgcctgtg gtgcctggg ctggagtg gctgtgga 15960  
 ccccccctc ggggaggg ttgctggc totgtgga ccccccctt tcccccggc 16020  
 CCGCATCGCC GGCACAGCA GCATGATTG CATGACATG CTGATGAGA ACATGTCAC 16080  
 CAAGgttag gggccctgg ggtttggg gtgggggt agagccccc oggttggc 16140  
 cctgtcctg cctgtggt tgcctccc ccccccggc CTGACTGTG ACATTGACAT 16200  
 CAGAGAGCC ATCGATGCG TCGGGCGCA GCGCTCGCG ATGCTGAGA CCGAGCGCA 16260  
 GTACAGTTG ATCTAGTGG GCATCGCGA GTTGATTAA AGCACTAGA AGAAGCTGA 16320  
 GTCTCTGAG gtgtcag agcagcct gggggggg ggggagtg tccagagtg 16380  
 gtgcctcgt ggcctgtgg ggcacccc ttcccctgt cccctgtgg acagtccag 16440  
 AAGGGCAGG AGTGGAGTA CCGGAGATC ACCTATCCC CAGCATGAA GAATGCCAT 16500  
 GCGAAGGCT CCGCAGCTC GTCCAGtg gtggcctga ctgcacgtc cgggctca 16560  
 ccccttgt ctcgcagcc cgtctccc ttctggga gggcaggtg tgggtggg 16620  
 gggagctgg ttaagttc ggttgttg tcccccctt tttcttgg ccttctga 16680  
 gtgcacccc gtgtgctt ctgtaggta ccagcagcc cctgtgtat gagggtag 16740  
 cctgttcc tggagcttg ggtgtagt caggccctt ggtgtgtc cctgtgga 16800  
 ggggtgga gggggagt ccagtgagg gcccctgt ggtgtctt ggcctgccc 16860  
 aactgctgt acttcccc ctgcaccc ctgcagCAC AAGGAGATG TGTATGAGA 16920  
 CTTGACACT AAGACAGA GGGAGAGAA ATTGAAGAA CAGCGTCCG CAGAGAGGA 16980  
 GAGAGAGAG GGTTCCTCA AAGGAGGTG AGCGTCTCT TCTCAGGTG GCGATGtcc 17040  
 agctctct cctgggtg cctccctgc tgcctgtgt cctgtgtcc actgttcc 17100  
 ctggttgt ggtgtggc cagctcatt ctgtgtcc cagctgccc agacccctt 17160  
 gtgcaccc caggttccg ctacccctt actccctcc tccctctct tggcagCTC 17220  
 AGCCCTGAG CTGTGAGAG ATTGCGGAT GACAGACTC AGAAGCTGA CTAAGAGTG 17280  
 GCGATGCTT TTGTAATTG AATGCTGCA TCCGCCGAC CTCTCCCTG CCGTBTATAT 17340  
 AGCCGAGCA AGGCCAGGC AGGGCAAGC CTCTCTCTT TGTAAATAA GCGCTGGAT 17400  
 CACTgtgt gctccttg ccttgtgt ggcagtag tggccgga ggggaggg 17460  
 caggtgtgt aactgtgt ggcctgtg gtgcctgg tggagctcc gcttccgt 17520  
 agagaggt ggttgggc tccctctg agtgagg gtgcctgt gtgcctcc 17580  
 cagctctt tttttaa gggagctt cctccagtg tgggtgga aggcacag 17640  
 gtgggtgt aggtagag gggcagtg actccccc cgttgcct gtgcacag 17700  
 actaagag tcccccgt agtgctgt aggccttct gctcttca tttccctg 17760  
 gggagctg agggagag aactagtg actgttcc ttgcccag tccaggtt 17820

# [Drawing 10]

|  |       |
|--|-------|
| utggacact gaggctgga ttgaccca ggtgtctga cccagagac oacactact   | 17880 |
| moontgagt tgcagctgg gccacccca gggggccot gatcacact oactgtgt   | 17940 |
| gatttccga ttgaccta gaggagtgt taacagcgg agagccagc ctgaggccag  | 18000 |
| cccgctgag tccctctgg cgggacagg gacaggctcc tcaaggccc cgggacagc | 18060 |
| cagctctac cctactag gccgtgtg cctttactc attgagaga gtttgaag     | 18120 |
| ctgctgag tgaagatgc tttgttttt gtcctctct cttccccc igtcaggat    | 18180 |
| gggtttctt cttttttt aacactgtg tctggggg taagagcgg gggagtcac    | 18240 |
| cctcaggag tctgtggcc cactctgga aggtgtgga cagacaggt cccacagcc  | 18300 |
| agaggtggg cgtgactac tcaaacact cttacagag ccccggtgt aagagctta  | 18360 |
| acgttcccc gactctgtg accttagag gctgtcagg gaga                 | 18404 |

# [Drawing 11]

|  |      |
|--|------|
| tctctctac cagctctca atttccaga gtcggggg ctgtactca ctgtacgac   | 60   |
| ggagctgtg taaggattg ctgactatg cagccccc cttgtggtt tgggactgt   | 120  |
| gctgtctag cctttccag attggggcc agcacttct gaggatgac tcccccagg  | 180  |
| ttttctccc aggacccag ttttttact aagagaga cccactctt gactggggg   | 240  |
| agggagagg ggaacaaaa caagcactc ttccagtag cagctcttc aactctgac  | 300  |
| caagagata agggagcag cggctggtt gagggggga gttgggtg cccggtgt    | 360  |
| ctgaggagc tctcctgtt cccgacagc gggggcaga cgggtgac tcaagctga   | 420  |
| gcttcaggt gttactact ctttttatt cagctctga actagactc agggaggtg  | 480  |
| gctcaggac ccttgaggg tggccccc tgcactcca ggttaaggg tggggctct   | 540  |
| gggtcagc agctgggtt cagctccag cctcctgtt cactagcct gtagcttga   | 600  |
| gacgtgac aagtactta gtttttgt cctcagttt cggccaggt aactgaga     | 660  |
| tgaagaggt gactagctg aactacgtg tctgtcttt tagtgcagg gcaactgca  | 720  |
| agctgtgtt gactcctgt ggcgtctta tctcagcag tctctggtt gtttttcca  | 780  |
| cccccctgt tctgtctgc cttccttta aagagaggc ctgggagct tcccccagg  | 840  |
| tcccccctc cctgtgagg cggagtcag ccccccctc gctgtgagg agggaggt   | 900  |
| tccagcagc gacgacagc agggacccc agttccctc cctgcctgc cctgtgagc  | 960  |
| ccacttact gggacagca aggtctcag ggcacccc AGTATCCCA GGGCTTATT   | 1020 |
| TACAAAGGA GAAGGTTGG CCTGCTCG GGCCTGCTG GCTATATAC AGGTCAGGG   | 1080 |
| AGAAGTGGG GGAATGAGC GATTAAATT ACAAAAGAT GAGGCACTCC TAGGTTGAG | 1140 |
| TTTGTAGTG GTCCATCGG AATGCTTC ACAGGCTAG GCTGAGGct gcaagaga    | 1200 |
| ggagctgag gactgagg gtagctgga cctggagtg gacacaggc gctggggca   | 1260 |
| gtgggagc acagagtag gctggggcc cccactcac caggagagg cagtggagc   | 1320 |
| agggacacg gggaggcag ggggacccc caggacagc agctgtacCA TGGCACTG  | 1380 |
| AGGACAGCA CACTCACTC CTCTGAGGG AACCTTCT CTCTCTCTG TCTGCTGAC   | 1440 |
| CTCTCTCTT CACTTTCTC TCCCTTTCT TCTTATGTC CAGGTTTGA TAGACCTCT  | 1500 |
| CTTGTGTct gacgaggtt gacgggggc aagtacagg agttgtgca ggtcaggag  | 1560 |
| cagacacgg ggaacggca ctggagttc cctgtgcca cccgtctca cagtgaagc  | 1620 |
| agcacagtc cctgactag actcaggtt cctagagga agaggtaca tctctacac  | 1680 |
| gagtgtctg ctgtactca gacaggccc aacgttgg gcactcagg aagtattatg  | 1740 |
| acagagagg gtagaccca agctgaact tgaagccag tcccccagc tgcacactt  | 1800 |
| gtctctccc gaaagtggg atgggctgg gaggagaaa ggggtggtg cgggaggtg  | 1860 |
| gagtcaggg cactcactT GGAAGAGTG CAGGAGGCT TGGATGGGC ATTCTCATG  | 1920 |
| GCTGGGAGT AGGTATGTT GGGATCTCC GACTGCTGC CTTCTGCA cttaggac    | 1980 |

# [Drawing 12]

agggaaagt ggaagtggt ggtocagaa gggcaagt gacacatoc tgcactgag 2040  
 oooooooon oooooooon tgcotgnao gaoCTGCAH SACCTGCAC TTCTTTTAA 2100  
 TGATTCTAAT GAACCTGAGC ATGACACAT AGATGAACCT GTACTGAGC TCGCTGCA 2160  
 CGATGCGCA GCGCTGAGC CGACACAT GTATGCTCTT CTGATGCA ATGTACACAT 2220  
 CCAAGCCTgt gtagggaga gtcaaacoto aggtocaggo atagagagac acagagggag 2280  
 tgcotacoc oooooooon aoooooaggt goocotacoc GTTGTGAG ATBTGTGCA 2340  
 TGACGATGT GATGACATG ATGATGCTG TGACGCGAT GCGGCGGtg ggaagagag 2400  
 gggatgcat cagagagag toagagoto oooooagag gggatgaca gggatgact 2460  
 oagagagag gaoocagag agagagotga gctocagtg atggagaga atgtgocag 2520  
 ggtocagag caggtgtga tggaggttg oagagotgt cgtggagag otcacotog 2580  
 ggtotgag tgcagaggt ggtocagag ctgacagat ccttccotc toacototo 2640  
 gggagagct ctcactcta gtgagtggt goocacocac cagagagat ggaacococ 2700  
 autocacac ctgtctgac ggtocagag taocotgag otcocococ totocotga 2760  
 aggttatag aggtctoto aggtgtgtt caottocot ttocotcag cctgagag 2820  
 agggagag tagocotgag acagagaggt gggagagag aggtgagag gactagagag 2880  
 agcagtgag tagaggtt ctctctctt coagagtg gaaagagag otaggagag 2940  
 oocagagoc acotcagat gagocagag agotgagac ctagagag cgtctaat 3000  
 toaaagag atgtacac ataacococ oacacact ccttctgta aggtctaat 3060  
 aagagagag ggtcttaca gaagtgtg atgtgtg tagagagag cagagagag 3120  
 agctctgag gtagagag ctctgagag agcagagag agagagag oocagagag 3180  
 oocagagag gacotcag attcagagag ctgagaggt cccagagag acagagag 3240  
 gggagagag aggaagag aggtctgag aggtgagag toocagag gtagagag 3300  
 ggaacagag aggtgaggt oocotcoc ttctctgag ctagagag ttactaat 3360  
 agtaacag oocaggttt gaggagag ggaatcag oocacagat aggtctaat 3420  
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 aggttagag atgtctca gacacagag ctagagag gaggagag goococag 3540  
 ootctctca oocagagag ggcagagag gtagagag ctagagag cgtctctc 3600  
 ctagagag oocotcag acotcagag aggtctctc atcacacac tgcagtag 3660  
 tagagagag aggtctctc agcagagag aggtctctc otcacacac cagagagag 3720  
 ggaacagag ggaacagag ggaacagag ggaacagag ggaacagag ggaacagag 3780  
 aggtctctc ggaacagag ggaacagag ggaacagag ggaacagag ggaacagag 3840  
 tgcagtag ggaacagag ggaacagag ggaacagag ggaacagag ggaacagag 3900  
 aggtctctc ggaacagag ggaacagag ggaacagag ggaacagag ggaacagag 3960

# [Drawing 13]

ggtocagag tootctctc ttctgagag ggtgagag gtagagat cagagagag 4020  
 ctctgagag ggaacococ ggaagagag cagagagag ctagagag oocagagag 4080  
 oocagagag tggagagag aggaacocac ggaagagag ggaagagag gaggagag 4140  
 aggtctctc ctagagag ttctgagat aggtctctc ggaagagag aggtctctc 4200  
 tootctctc ctagagag atcacagag tootctctc CTGATGCA CGATGAG 4260  
 CGCTGCTGA GCGACATTT CTGCTGCTG GTTGTCTG TCGAGAGAG TGAGGACAC 4320  
 CGGAGCTGA CTGAGAGAG CATGCTGAG CAGCTGAG TACTGTAT GCGATCTC 4380  
 CGGATGAG TTCTCTag cagagagat aggtctctc oocotcoto totagagag 4440  
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 GTTGTATTC GTTGTCTGA TGCTCTCTC AGTTGTCTC AGGTAGAG CGATAGCAG 4620  
 GTGCTGAG CAGCTGAG CAGTATGAG CGATTTGT ctagagag aggtctctc 4680  
 ggaagagag oocagagag ggaagagag oocotcag gtagagag aggtctctc 4740  
 gtagagag oocagagag atgtctctc ggaagagag gtagagag gtagagag 4800  
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 oocagagag cgtctctc toacacag ttgagagag aggtctctc oocagagag 4920  
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 gtagagag agagagag cagagagag cagagagag aggtctctc ctagagag 5040  
 aggtctctc ctagagag cagagagag cagagagag gtagagag gtagagag 5100  
 tootctctc tootctctc ggaagagag gtagagag gtagagag gtagagag 5160  
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 ctagagag gtagagag aggtctctc gtagagag gtagagag cagagag 5280  
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 aggtctctc gtagagag aggtctctc aggtctctc aggtctctc gtagagag 5460  
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 aggtctctc gtagagag aggtctctc aggtctctc aggtctctc gtagagag 5640  
 oocotcag gtagagag ttctctctc gtagagag gtagagag ttctctctc 5700  
 gtagagag aggtctctc gtagagag gtagagag gtagagag gtagagag 5760  
 CTCTGAGAG GTGATGAG TGACAGAG GTTGTCTG CAGGATCT GCGAGAG 5820  
 ATTACAGAG GCGCTGAG AACCTGAG GCGATGAG GTCTTATCT TCTCATGAG 5880  
 GCGTACAG TGTTTctg aggtctctc gtagagag oocagagag gtagagag 5940

# [Drawing 14]

|  |      |
|--|------|
| tgcccccga caggccctga accactgca cccctaccca tgcagagagc agcttcggc       | 6000 |
| tttgccacga tgtctctgga totagggtcc ccccccacga caggagaitc ccaggagccc    | 6060 |
| gcctctctc cccagtggcc cccactgctg accttgaatg agttgscatt gatgtagtgg     | 6120 |
| gacccgagga tgttactgtc cctgtccctgc aggatcactc ggcctgtgtc aactggaggt   | 6180 |
| ggggagagag gaggccagat ggtctgggtt agccaggagc tcccacccc tgggtcagac     | 6240 |
| ccctctgagc taccaggcca ttctggtctc agagagccatg aggtttggg gggactgtg     | 6300 |
| gcagccaggg tggggatca totgtatgga gggagtggg ggttccaaa ggggttggg        | 6360 |
| agccaggagg accctgctg ctggtgtgg agaggacatt ctggaggag tagaggcatt       | 6420 |
| ttggagagga gggagctct gtggggctg tgggagatg gccctctctg ggtggaggg        | 6480 |
| tggagccctg tggagtggg gggagcagct agggccggg cgggtatctg ggtgagtg        | 6540 |
| gcagccctgg tgcacagag ggaagatggt ctgtatagcg ttcttgcctt tgttctctgg     | 6600 |
| ccctctgcct tgcacagcct ggtgcaagtt ctgcacctgc tgccttgcga aactctgga     | 6660 |
| tgtggtggg gactgagca tccgtccca agaggagct cactggggg agctccagt          | 6720 |
| gggtggctca gggccaggg tgggcaagga agggaggtg tccgctggg gccccagggc       | 6780 |
| gctgcaccca cctcagctg cccagccctg ccggtccca cactgacct caaactcctc       | 6840 |
| ccagagagca gcttgcctg tatcctcaga ctgctgctt ttgttcaatt ccaagactcg      | 6900 |
| gttctcaatg tgcagcccat tgcagcctgt ggcataatag gcttggctg gggagaggg      | 6960 |
| ggagtcagc aactcagga tccagagagc tccactctg gggcagctc agggaggtg         | 7020 |
| ggggagcagc tgggcccac cctgacctgc ccgaggtaga caaagccgac tgagccctgc     | 7080 |
| tcaatccccc ttttcttgaa atgctccacc aggtccctga gcttctgaa gctgtccaaa     | 7140 |
| ccagccactg tttagcctgc agctggggg gggcccaatt aggcattca caggcctga       | 7200 |
| gtctccctcc ctgggtccc agcttgact tccagccag gggccctc cctgcaccc          | 7260 |
| accctgtgt cccagaggg agcttcagca gggctcccc agccgcttg ctgccttacC        | 7320 |
| tccacacatga ccttgaatga ggtgacccctg agccggagac ctgggcccagc cttgggctgg | 7380 |
| tcactgagca caaaagccac gaattctcca gctgagctga gctctcagc cacaagaaac     | 7440 |
| gtcgagagct cggccttgag ctgacagcag gttcttgcct gcccgccaga catgtggcca    | 7500 |
| tggtagcac tagagaggg aggtcaggg aggttaagc ccaggcccca ggggagggc         | 7560 |
| ctgagcagc acattcaag agagggggg gaaatcagc aactctgtg tggcaggcc          | 7620 |
| tggctacat gttcccaat cccacacctc atttaggtt ctacacaaa tgcagagag         | 7680 |
| taggtatgat attaccccca ttttatagat gaggagctg agctgagct gttactcct       | 7740 |
| ggcttagggc ccttgaatga ctctaggcca agcttcaggc gctctgttc ccagctgga      | 7800 |
| gtgagtgga ggggttcaag ctctctgag cctagacctc ccaggttcaa gccctctag       | 7860 |
| ccctcagagt totacccgg ctctgacccc agagccccc tctgagagc agagaggctg       | 7920 |

# [Drawing 15]

7980  
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9840  
9900

|             |             |             |             |             |            |       |
|-------------|-------------|-------------|-------------|-------------|------------|-------|
| agagcagagat | ttoitgtgat  | tootaggagat | taagttagaa  | agagtagtag  | ttagctotg  | 9960  |
| agagcagagc  | toocagagca  | gagagagaga  | ggagcattga  | gagcagagag  | taagttagat | 10020 |
| agggagagag  | atggaggata  | ggagcagagc  | tootcagcto  | toactgcto   | tootggctgt | 10080 |
| agagtagtag  | gagctaatat  | oatgtgtgat  | gagtagtagag | gagtagtagag | oatgaatgct | 10140 |
| gactagttag  | atgagagaca  | ttcagcagag  | acttagagat  | gagtagtagt  | oatgttagct | 10200 |
| gcttagagag  | agcctgctgct | tgaatgtgat  | atcagcagag  | ctgtgttaga  | gtcagagagc | 10260 |
| ttctccctgc  | atcagagctg  | tagaaatgct  | ctgagttgtg  | gagcagagat  | taggttagct | 10320 |
| gatcccgac   | ctccagagag  | oagagagagc  | gagtagtagt  | gagtagtagt  | gagtagtagt | 10380 |
| agggagagga  | taggtgtgtg  | tootgtgtgt  | ggagcagact  | ggagtagtag  | ggagtagtag | 10440 |
| agagtagtag  | agtgtagtag  | ttggagagga  | ggagtagtag  | ggagtagtag  | oatgtagtag | 10500 |
| ggagagagag  | agatgtgtgt  | taagagtagt  | gagtagtagt  | gagtagtagt  | agagtagtag | 10560 |
| ggatgtagag  | ggagcagact  | acttagagca  | gagtagtagt  | gagtagtagt  | ttgttagtag | 10620 |
| ggagcagagc  | agagagagct  | ctgttagagc  | toagcctgt   | agcagagct   | ctgttagtag | 10680 |
| gcactgtgag  | gttgcctgaa  | acacagtag   | agggagtag   | agggagtag   | ggagtagtag | 10740 |
| cgtagtagag  | agcagagctg  | ctgcctgtgt  | taagctgtgt  | ggagagtagt  | taacttagca | 10800 |
| tccttagagc  | ttccagagag  | gagagagagc  | gagtagtagt  | gagtagtagt  | ctagttagag | 10860 |
| gagtagtaga  | gcttagagag  | taagctgtgt  | atgagctgt   | gagtagtagt  | ctagcttagc | 10920 |
| acccgcctgt  | gtccacagtg  | tgagctgtgt  | acttagtagt  | oatgtagtag  | oatgtagtag | 10980 |
| ggagtagtag  | agagagagag  | oagcagctgt  | ggagtagtag  | acttagtagt  | ctgttagtag | 11040 |
| agagcagact  | ggagagagag  | gagagagagc  | gttagtagtag | ggagtagtag  | ggagtagtag | 11100 |
| toocagagct  | ctgagtagag  | aggttagag   | gagtagtagt  | gagtagtagt  | gagtagtagt | 11160 |
| agattagag   | acttagtgtg  | oatgttagag  | toagtagtag  | gagtagtagt  | gagtagtagt | 11220 |
| agagcagagc  | gtccagagag  | ctgtgttagt  | oatgttagag  | ctgtgttagt  | gagtagtagt | 11280 |
| ctgttagagc  | ctatagagag  | tgatgttagt  | gagtagtagt  | atgtgttagt  | toatgttagt | 11340 |
| tgagcagagc  | tggtgttagt  | tggtgttagt  | tgatgttagt  | tgatgttagt  | ctagtagtag | 11400 |
| oatgttagag  | agagtagtag  | cttagtaggt  | ttagtagtag  | gagtagtagt  | gagtagtagt | 11460 |
| tttagagct   | tgcttagtag  | ctagtagtag  | tgatgttagt  | tgagtagtagt | gagtagtagt | 11520 |
| catgttagt   | ctagtagtag  | tgcttagtag  | toatgttagt  | agagtagtag  | gagtagtagt | 11580 |
| agagtagtag  | agagtagtag  | tgcttagtag  | ggagtagtag  | ggagtagtag  | gagtagtagt | 11640 |
| tagtagtagt  | oatgttagt   | gagtagtagt  | gggttagtagt | cttagtagtag | catgttagt  | 11700 |
| tgcttagtag  | oatgttagt   | gagtagtagt  | oatgttagt   | cttagtagtag | ctgttagtag | 11760 |
| gggttagtag  | taattgttag  | oatgttagt   | cttagtagtag | oatgttagt   | oatgttagt  | 11820 |
| oatgttagt   | ggagtagtag  | gagtagtagt  | cttagtagtag | cttagtagtag | oatgttagt  | 11880 |



# [Drawing 17]

|   |       |
|---|-------|
| tgttettaga ggcottnao actnaggtat atgntgtgo ctacotgtan ttocaaatac | 11940 |
| aaacattnao aocogonao oaaaaagag aaagagagot caacagggaa taacaaaga  | 12000 |
| aaacatattg gtggtttoo ctaggagagg aaactggtg gctgagagao aggtotggg  | 12060 |
| gagaaacttg gttototao taacttttg atgttatat gttgggagt acttaacttt   | 12120 |
| ataaaataa atgtctaac tgggaacat ttctgggaa cttttaggt acaaggagag    | 12180 |
| ctctttgtot ctatctggt gttgtttga tgggtgtga taacotgtan gtgggocgg   | 12240 |
| cgactggot cactgtcta atcocaagca ttgggaggo caggotggc ggtatogag    | 12300 |
| gtocaggagt agagacooto atggocaaoc tgggttaao cactototao taanaataa | 12360 |
| aaaaattag atgggagtg tgggtggag atgtatooo agotactgg gaggtagag     | 12420 |
| caggaganto acttgaaco aggaagocga ggttgaagag agocagato aococotgo  | 12480 |
| actocagot ggaocaaag taagatota tttaaaaaa aaaaaaana tggagocag     | 12540 |
| ttgtgtott actatttat aacttatga atgtctgt ctacttaaa gaagaaagag     | 12600 |
| ttttaagoc aaacagtota attttccat gataactaa ttatctttt tagaaacatc   | 12660 |
| aaacatcaat gttttccoc aaaaaggtt tttaacttt totttttto tttttgtot    | 12720 |
| attgtttot gttttctto tctocotgt gcotaggaa atctgtctg tgggtttat     | 12780 |
| gactgttaa gotttgaota oocotttago ttggaocaa oocagagao caggaaatoc  | 12840 |
| ggggaacoo cagococao goaaggago aagagagaa catgaagao tggctgaga     | 12900 |
| ggggaacoo agtggagot ctogotato toggagitt caggctacg tgoatgoot     | 12960 |
| ggaactgag aagtgttaa caaocagot ogtgaatoo tagcaacata cctggacaa    | 13020 |
| agtaagott cctgttota caococago ctgtttgta taagagagaa agggagaa     | 13080 |
| oacagttaa gaaacacaa ctactatoo atgtacact aacacatac ctgttcagt     | 13140 |
| gaaatocoo agaatgga actaatotg aococaaag aagttaagg toactgtaa      | 13200 |
| gaacaaag cacaotgao ootggocaa agaaatoot tocaggott aaacocact      | 13260 |
| atggagag aattaatog gaacocaga gaactaat agaacotot tootgaocaa      | 13320 |
| tggagaggo agaattoot atgaaggty taagocaaag agacagaact cagaaagag   | 13380 |
| gtacagotto tgotatcct tggagagaa ataaaaact aactoccta ccttggagt    | 13440 |
| gttggggtt tgaagtac tttaacatac agatctcat ttaggactaa gaaagocotg   | 13500 |
| tgtgtaga atcattttc cacttttct atggtagaa acaagatag ggaatuaag      | 13560 |
| aattacaga gtaaatgct gatacttag aaaaaaaan tatitcott gaagcagaa     | 13620 |
| gataaagta gatataag caggagcag tggctcagc atgtatccc agcacttgt      | 13680 |
| gggttagg atgtgatt acttgaac cagttaga gacactagg aaacttgag         | 13740 |
| aaacttgt toaacaaan atacaant tagaaagt tggtagocaa oocotgtag       | 13800 |
| ccaggtact tgaagact agatggag atogcttag cctgggtg tagagotgo        | 13860 |

# [Drawing 18]

13920  
13980  
14040  
14100  
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14220  
14280  
14340  
14400  
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14760  
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14940  
15000  
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[Drawing 19]

15900  
15960  
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16140  
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16260  
16320  
16380  
16440  
16500  
16560  
16620  
16680  
16740  
16800  
16860  
16920  
16980  
17040  
17100  
17160  
17220  
17280  
17340  
17400  
17460  
17520  
17580  
17640  
17700  
17760  
17820

# [Drawing 20]

|   |       |
|---|-------|
| gagagagagc ctoaggtagg gtttagccct ggggaggggt tagggccagc cagggaccata  | 17880 |
| ggagagagct gttcaggggt atggagcctg gaagggcaga gggagtcagg gaaggagaga   | 17940 |
| tgggggttgt caggtcagca agagagctgac mcccaggtta agtcacaggc gattttatta  | 18000 |
| ttgtatggaa cgtcagggcc ttctctatccc tggcaggtggo aagggggagg ggcacatctc | 18060 |
| agggaggggo cagctgcca cagcagggag ggcacatggz tggcaggtc atggcagggc     | 18120 |
| gaagcagggc cttcaggttg cctgaggggo tggcaggtc agcagctgg gcttcagtagg    | 18180 |
| actgagcga gagagagaa ttagggagaa ctgagagagg gaggagagg tccagccttc      | 18240 |
| tcctagggac cagctgctt agggagcctg gggggggttg tagctcagc atgagctgt      | 18300 |
| ccccccctgg ctggcagct gtttgcgaa caggagagat gggcagctt gtcagctgtg      | 18360 |
| gtttgagga tgcagcttg tctcagctg ggcagagat atgg                        | 18404 |

# [Drawing 21]

|  |      |
|--|------|
| ATGCTGTCC GTGGGTGTT TCACGAGAG CTCAGTGGC TGGATGAGA GACCTGTCT  | 60   |
| AAGGGCCAG GTGTGAGG TACTTCTG GCTGGGCCA GTGCAAGAA CGAGGTGAC    | 120  |
| TTCTGCTCT CGTCAAGGT GAGGATCAG GTGAGCATA TTGATGCA GAACTCAGG   | 180  |
| GATTTGTAT ACCTGTATG AGGGAGAG TTTGGACTC TGACAGAGT GTTGGATAC   | 240  |
| TACACTCAG AGCAGGTGT CGTGAGGAG GCGAGGGGA GATGATGCA CCTCAATAC  | 300  |
| CGCTGAAC GTCTGATCC CACTATGAG AGTGTATCC ATGACCAT GTCTGGCGG    | 360  |
| CAGGAGAGA GCTCTGCA GCGCAGGGG GAGCCTGGA GTTTCTTGT GGTGAGAGC   | 420  |
| CTGAGCAGC GTGAGAGTT GTCCTTTCT GTGTGAGTG ACCAGCCAA GGTGGGCCA  | 480  |
| GCTGCCGC TCAGGTGAC CCACATCAAG GTCATGTGC AGGGTGAGC CTACAGAGT  | 540  |
| GGTGGTTTG AGAGCTTGA CAGCTCAGG GACCTGTAG AGCATTTCA GAAGAGGGG  | 600  |
| ATTGAGAGG CCTCAGGCG GTTTGTCTC CTGGGGCAG CTTACTATG CAGGAGGTG  | 660  |
| AATGCGCTG AGATTGAG CCGAGTGTG GAATGAGAA AGAAGCAGG GTCCGAGAT   | 720  |
| ACAGCAGAG GTGGCTCTG GAGGAGTTT GAGATTTTC AGAAGCAGG GTTGAAGAG  | 780  |
| TTGCACAGC GTGTGAGG GAGGCGGCA GAGAGCAGG GAGAGAGCG CTACAGAGC   | 840  |
| ATTCTCCCT TTGACAGAG CGAGTGTAT CTGAGAGAG GAGAGATTA CATGCCGGG  | 900  |
| TGCGACTCA TCAATGCCA CTACATCAAG AACGAGTGC TAGGCTTGA TGAGAGCGT | 960  |
| AAGACTACA TGCGAGCCA GGGCTGTCT GAGGAGAGG TCAATGACT CTGGCAGAT  | 1020 |
| GCTGGCAGG AGAAGAGCG GTTCATGTC ATGAGCAGC GAGAGTGA GAAGAGCGG   | 1080 |
| AACAAATGG TCGATAGT GCGGAGAGT GGCATGAGC GTGCTTATG GCGTACTCT   | 1140 |
| GTGACCACT GCGGAGAGC TGACAGAGC GAATCAAG TCGTACTTT AGAGTCTCC   | 1200 |
| CGCTGAGCA ATGAGAGCT GATTGAGAG ATCTGCAAT AGCAGTACT GAGTGGCC   | 1260 |
| GACCATGGG TCGCAGTGA GCTGAGGCT GTCTCAGCT TCGTGAACA GATCAAGAG  | 1320 |
| GCGCAGGAA GTCTGCTCA GCGAGGCCC ATCATGTGC ACTGAGAGC GCGCATGAG  | 1380 |
| GCGCAGAGC GATCATTTT GATGAGAGT CTGATGAGA ACATCTGAC CAGGGGCTG  | 1440 |
| GACTGTGACA TTGATGCA GAGAGCATC GAGATGTGC GCGGAGAGC GTGGGCTAG  | 1500 |
| GTGAGAGAG AGGCGAGTA CAAGTTGATC TAGGTGGCA TCGCCGATT CATTGAAAC | 1560 |
| ACTAAGAGA AGCTGAGGT CCGCAGTGC CAGAGGGCC AGGATGAGA GTACGGAGC  | 1620 |
| ATGACTATC CGCGAGCAT GAGATGCT CATGGAGG CCGCGGAC CTGCTGCAA     | 1680 |
| CACAAGAGG ATGTGTATGA GAGCTGAC ACTAAGAGC AGAGGAGGA GAAAGTGAAG | 1740 |
| AAGCAGCGT CAGCAGCAA GAGAGAGAG AAGGTTCC TCAAGAGAA GTGA        | 1794 |

# [Drawing 22]



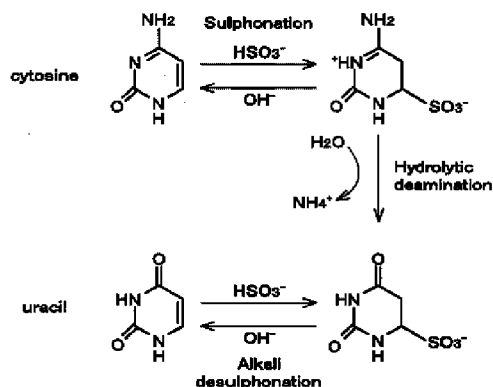
# [Drawing 23]

MVRWFHRDL\$GLDAETLLKGRGVHGSFLARPSRKNOGDFSLSVRVGDQVTHIRIQNSG  
 DFYDLYGGKEFATLTELVEYTTQQQVLQDRDGTIIHLKYPLNCSDPTSERWYHGHMSG  
 GQAEITLLQAKGEPWTFVLRESLSQPGDFVLSVSDQPKAGPGSPLRVTHIKVMCEGGRY  
 TVGQLETDFSLDLVEHFKKTGIEEASGAFVYLRQPYATRVNAADIENRVLELNKKQESE  
 DTAKAGFWEEFESLQKQEVKNLHQRLLEGQRPENKGNRYKNILFPDHSRVILQGRDSNI  
 PGSDYINANYIKNQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRRVIMTTREVE  
 KGRNKCVPYWPVEVGMQRAYGPYSVTNCGEHDTEYKLRTLQVSPLDNGDLIREWHYQ  
 YLSWPDHGVPEPGGVL\$FLDQINQRQESLPHAGPIVHCSAGIGRTGTIIVIDLMLENIST  
 KGLDCDIDIQKTIQMVRAQRSGMVCTEAQYKFYVIAAQFIETTKKKLEVLSQKQGESEY  
 GNITYPPAMKNAHAKASRTSSKHKEDVYENLHTKNKREEKVKQKRSADKEKSKGSLKRK

# [Drawing 24]

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| actocstott | gocggtgtoc | ttaagtgtg  | tcttggtc   | agtcotc    | agcgcgcga  | 240  |
| toctgagat  | ccagcctgt  | cagcagcgt  | gagcctg    | ttctgc     | ccatgaccc  | 300  |
| gocaccccat | ggcctgtgt  | ctggtgcgc  | ctgtgc     | cc         | toctgagat  | 360  |
| ttgtcccat  | gocagggag  | gagcgcct   | gctgtccg   | acatctaa   | atgctgt    | 420  |
| gaottagcct | tggtgtagt  | ctcttgga   | cctgacac   | ccatctctc  | cttccctgt  | 480  |
| toctctgoc  | tttccagcc  | ccatccccc  | gacagctoc  | toctctggt  | cctgctggg  | 540  |
| ccatccctg  | cccccggcc  | taacccatc  | tgagctcct  | ccctctccc  | ggcgcaggt  | 600  |
| tgagcagct  | gctgggtcc  | ctcagcc    | tggtggg    | gctgcac    | ggcgtcc    | 660  |
| ccctctgga  | gacctggc   | tggtaggat  | tttccctag  | cccttggt   | tcctcctc   | 720  |
| gagcgttc   | cccatgtgt  | tgctctcct  | cagccaggt  | tactctctg  | ctgttccc   | 780  |
| taacccatc  | ccctctc    | tgctagctg  | agctccaggt | gagctccg   | gtgctccct  | 840  |
| ctctcc     | ggcgcgc    | cctgacac   | cagctgc    | tgctgctc   | ctcttggt   | 900  |
| ctgcagcga  | agctcc     | tggtgct    | ctgctcc    | ccctccca   | cctgtcctt  | 960  |
| toctggagc  | tattgtcc   | gctgtgtc   | ctgagctcc  | ATTGCGTGG  | CAGGCAGAT  | 1020 |
| CAGGAGGAA  | GTGCTGATT  | ACTGAGCGGT | TCTTCTCAC  | CTGCTTGGG  | CCAGTGTGA  | 1080 |
| CAGCTGTGCG | GCTGCTGAG  | CCGCGCGCG  | TGCGCCCTC  | CTGCTTGGT  | TGCGCGTCC  | 1140 |
| TACAGAGABA | TGCTGTCC   | TGGtaagtc  | cctgcacca  | tgctgctc   | agctccctgt | 1200 |
| tgcttttga  | ggcgcgc    | gctgtgtga  | tgctccctc  | tgctgtg    | agctgagtg  | 1260 |
| ctctctg    | ctgcctc    | cctgttcc   | gtccctatga | actccctt   | ccctgaggt  | 1320 |
| gtgagcccc  | ctgctcct   | cctgtcctc  | tgccctctc  | ttaacattt  | ccctgacac  | 1380 |
| agctgtctc  | tgctctcc   | atgctctc   | tacttccgc  | ctctggctc  | ctgcttctg  | 1440 |
| ctctgctta  | ggcctgtcc  | ccctgggtg  | ctccacac   | ctccacata  | gagctcagc  | 1500 |
| goccc      | agcgcctcc  | ccctccgc   | ccctctc    | ccctccca   | ccctccct   | 1560 |
| ttggtcccat | ctctttgtt  | tocttccct  | cccttcc    | ctgctccct  | ccctccct   | 1620 |
| gtcctgtt   | agctctctt  | ctgcacac   | tgctgttcc  | gctgtgtta  | atctggagt  | 1680 |
| gacccagcc  | gacgtgtct  | ctgctctcc  | gagcttcc   | ctccctggg  | agcttccgc  | 1740 |
| ctccacac   | acccacata  | atggtctct  | tgagctct   | cagctgtat  | ccctgacac  | 1800 |
| tgacagctc  | tgctgtg    | tgctgttcc  | ccctgtgt   | ccctgctc   | tgctgggtg  | 1860 |
| agcctgagc  | atgctgtg   | ccctgggtt  | tgacccgc   | ctgctccata | tgctgagcc  | 1920 |
| ctgtctctc  | acccacata  | agctgtgt   | tgctgtgtg  | ccctctc    | tgcttccgc  | 1980 |
| ctgtggagc  | gctgaggtg  | gagctgtgt  | tgagctgtg  | agctccgc   | tgctgagcc  | 2040 |
| gctgtgtga  | ccctgacac  | ccctgtgtg  | ccctgagc   | agctgtgt   | ccctccac   | 2100 |
| acccacata  | gacgtgtc   | cagcttctc  | tgctgagc   | tgctgtgtg  | tgctgtgtg  | 2160 |

# [Drawing 25]



[Drawing 26]



[Drawing 27]

|   |      |
|---|------|
| ttttttttttt gttttttttt tttttttttt aatttttttt aatttttttt | 60   |
| ttttttttttt tttttttttt aatttttttt tttttttttt tttttttttt | 120  |
| ttttttttttt tttttttttt tttttttttt gttttttttt aatttttttt | 180  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 240  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 300  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 360  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 420  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 480  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 540  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 600  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 660  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 720  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 780  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 840  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 900  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 960  |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1020 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1080 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1140 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1200 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1260 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1320 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1380 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1440 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1500 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1560 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1620 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1680 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1740 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1800 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1860 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1920 |
| ttttttttttt tttttttttt tttttttttt tttttttttt aatttttttt | 1980 |

# [Drawing 28]

|   |      |
|---|------|
| ttatggag gtaaggtg gaggttgt taagttag agttaagt ttagttag   | 2040 |
| gagttgtg ttattgata ttatttag gtttaggt agttttgt ttatanaaa | 2100 |
| aaaaaaaa gaaatgat tattttata ttatttag ttattgtg ttaggtgt  | 2160 |
| atttttgt gtaggtta gtaggttt tttagggg ttatttga gtttagtt   | 2220 |
| gtagggag gtaggtgt ttattgtg agtttgtt taagttaa gttttttg   | 2280 |
| gttaggag ttattgtat aagtttta tttaggaa ataaaaaa gtaggtgt  | 2340 |
| ttattgtg aatatttg tttagggg ttatttat agtttagg ttattgtg   | 2400 |
| agtttgtg tttaggtg aattttag agtttgtg gtttagtt gtttttag   | 2460 |
| ttttttgg gtttaggg gtaggtgt ttatttag gtttttaa ttatttag   | 2520 |
| taatttag agttttat ttatttaa aatatttg gtttgtg tttaggtg    | 2580 |
| tttagttt agttttg gtttgtg tttaggtt tttaggtt aagtttta     | 2640 |
| gatttttg gtttaggt gtaggttt ttttttat agtttagg ttatttat   | 2700 |
| ttatttaa ttattgtg ttattgtg ttattgtg ttatttat tttaggtg   | 2760 |
| agtttagg attatttg tttaggtg agtttgtg gtttgtg tttaggtt    | 2820 |
| gtttttgt tttaggtg agtttgtt ttgtttaa aaaaaaaa aagtaggg   | 2880 |
| gtagggag gtagggag gtagggag aagtagga gtagggag aagtaggg   | 2940 |
| gtagggag gtagggag gtagggag aagtagga aagtagga aagtagga   | 3000 |
| agtttat tttaggtg aagtagga aagtagga aagtagga tttaggtg    | 3060 |
| aaaaaaag aagtagga tttaggtt agtttgtt aagtttta tttaggtt   | 3120 |
| tttaggtt gtaggtg gtttaggt tttaggtt aagtaggt tttaggtt    | 3180 |
| gtaggtt tttaggtt aagtagga aaaaaaat agtttgtt agtttgtt    | 3240 |
| tttaggtt tttaggtt agtttgtt agtttgtt tttaggtt tttaggtt   | 3300 |
| gtaggtg gtaggtg tttaggtt tttaggtt tttaggtt gtaggtg      | 3360 |
| tttaggtt aaaaaaaa aaaaaaaa aaaaaaaa tttaggtt agtttgtt   | 3420 |
| gtaggtt gtaggtt gtttttgt tttaggtt tttaggtt tttaggtt     | 3480 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt   | 3540 |
| tttaggtt tttaggtt gtttgtt tttaggtt tttaggtt tttaggtt    | 3600 |
| tttaggtt agtttgtt tttaggtt tttaggtt tttaggtt tttaggtt   | 3660 |
| gattttat tttaggtt aatttgtt tttaggtt gtttgtt gtttgtt     | 3720 |
| tttaggtt gtttgtt gtttgtt tttaggtt tttaggtt tttaggtt     | 3780 |
| gattttat tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt   | 3840 |
| gatttgtt tttaggtt tttaggtt aagtttgt agtttgtt gtttgtt    | 3900 |
| gatttgtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt   | 3960 |

# [Drawing 29]

|   |      |
|---|------|
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4020 |
| gatttgtt agtttgtt aagtttgt agtttgtt tttaggtt tttaggtt | 4080 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4140 |
| agtttgtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4200 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4260 |
| gatttgtt tttaggtt agtttgtt gtttgtt gtttgtt tttaggtt   | 4320 |
| tttaggtt gtaggtg gtttgtt tttaggtt tttaggtt tttaggtt   | 4380 |
| agtttgtt gtttgtt tttaggtt tttaggtt tttaggtt tttaggtt  | 4440 |
| gatttgtt gtttgtt gtttgtt tttaggtt tttaggtt tttaggtt   | 4500 |
| tttaggtt agtttgtt tttaggtt tttaggtt tttaggtt tttaggtt | 4560 |
| tttaggtt gtttgtt tttaggtt tttaggtt tttaggtt tttaggtt  | 4620 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4680 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4740 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4800 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4860 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4920 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 4980 |
| agtttgtt tttaggtt tttaggtt agtttgtt gtttgtt gtttgtt   | 5040 |
| gtttgtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt  | 5100 |
| gtttgtt tttaggtt gtttgtt tttaggtt tttaggtt tttaggtt   | 5160 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5220 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5280 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5340 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5400 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5460 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5520 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5580 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5640 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5700 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5760 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5820 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5880 |
| tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt tttaggtt | 5940 |

# [Drawing 30]

|   |      |
|---|------|
| ggttggtgt aatttgggt ttttgggtt aagtgtttt ttgtttta tttttoggt    | 6000 |
| agttgaggt ataggogtt attatttgt ttgttaatt tttttgtt tttagtagg    | 6080 |
| aggggtttt atttgttgg ttaggatgt tttgttttt ttatttogg atttgttgt   | 6120 |
| tttgttttt taagtgttg gatttatgg ogtagttat ttogttoggt taattttag  | 6180 |
| tttatatata tttagttaa tagtatttg atagatata agatttttt ttgtattta  | 6240 |
| aaagttttt agaatgtgt tttagtttgt atatttttt ttataaagt atgtatgtt  | 6300 |
| tatttatata tttttaaaa gttatgtag gaatttagt ttttttttg ttgtttttt  | 6360 |
| tagttattta gtttttttt ttaggagag ttattatatt gtgttttta tgtattttt | 6420 |
| gttagttat ttttttgt ttgttttg oggttgtgt gtgtttttt ggaatttag     | 6480 |
| gtagtata ttatatatt ttgttttg gtttttttg attatttgt tttagaaaa     | 6540 |
| ttagttag ttagggttt gtttttgt ttttagaga tagtttttt gtttaggtg     | 6600 |
| ttgggttag gttaggttt aggtataga attagaaag atttatttg atagggtgt   | 6660 |
| ttagggtat gtgtttatt ttttttgt agtttagt ataattgta ttgttgtgt     | 6720 |
| agggaggtt atttttttt ttgttagta ttgttaggt tttaatttt agtttttgt   | 6780 |
| tttttgtta gtttttttg ataggaggt tttaatttt ttgttttga agttttgag   | 6840 |
| gttgttat aggtttta agtattgt ttgaatogg atgttttgg ttgaatttt      | 6900 |
| gttgttag ttgtttatt gttgtttta gttatgttt taatttttt ggtttttag    | 6960 |
| tttttgttt gtaaatgtt aaagtatgt ttgttttat aggttttgg ttgaattta   | 7020 |
| attagatag gtatgtaga gttatttag tatagotto gttatttgt aggtttatt   | 7080 |
| oggtatag ttatttgt attatttta tttagtgg ttaggagga tttagttaa      | 7140 |
| gtatttgtt gaggagaga aggttgtt gttatgttg gtttgtat ogtagatgt     | 7200 |
| tatttgtgt tttagtgtt aggtatata ttgttttta ttgttgtgt ttgttatat   | 7260 |
| gttaattgt atgttttga gtttttgt ttgaatgtt gtttagtgg ttgtttttt    | 7320 |
| atttttagt ttatttgtt ttgtttttt ttttttgt ttgttttaa oggaagtat    | 7380 |
| aagtgtttt ttttaaaa ttgttgtgt ttttttgt ttgttttg ttgttgttt      | 7440 |
| tagttatgt GATTGATG TTTAGAAATG GAATTATCG GGTGATGAG GCGTTGCGT   | 7500 |
| ATTGGAGTT GTATTGAGG TTTAGTTTTT GATTTTTTT TTTGTTAGA TTATTGTAT  | 7560 |
| TTTTTTATT TTTCGTTTT TTTTTTTTG GGAAGTTTT AGGATGGTGA Gtaagggtt  | 7620 |
| ttttattac gtttagtag aggtagggt gtttgtgt ttogggttt tttttattg    | 7680 |
| tttgttttg ttgttttag TGGTTTATC GAGATTTAG TGGTTGAT GTAGAGATT    | 7740 |
| TGTTAAAGG TGAGGTGTT TACGATAGT TTTGTTTCA GTTAGTGT AAGAAATAG    | 7800 |
| GTGATTTTG GTTTTGTTT AGtaggtg gtttttgt atttaggtt ttgttttat     | 7860 |
| tttttgtgt ttttaggt ttgaattat ttttttgt tttagtgt agtttgttt      | 7920 |

# [Drawing 31]

7980  
8040  
8100  
8160  
8220  
8280  
8340  
8400  
8460  
8520  
8580  
8640  
8700  
8760  
8820  
8880  
8940  
9000  
9060  
9120  
9180  
9240  
9300  
9360  
9420  
9480  
9540  
9600  
9660  
9720  
9780  
9840  
9900

[Drawing 32]

9960  
10020  
10080  
10140  
10200  
10260  
10320  
10380  
10440  
10500  
10560  
10620  
10680  
10740  
10800  
10860  
10920  
10980  
11040  
11100  
11160  
11220  
11280  
11340  
11400  
11460  
11520  
11580  
11640  
11700  
11760  
11820  
11880



[Drawing 33]

|            |           |           |            |            |           |       |
|------------|-----------|-----------|------------|------------|-----------|-------|
| atagttttt  | ttttatgt  | tttttttt  | ttttatgg   | ttattttt   | agtttagag | 11940 |
| aggtttttt  | ttttatttt | tttagagtt | tttttttt   | tttaaaagt  | tttttttt  | 12000 |
| ttagaaggt  | tttttttta | ttagtagta | ggtttttt   | tttttttt   | ttttttga  | 12060 |
| atttttttt  | tttttttta | tatagtatt | tttttttt   | ttgtttatg  | ttttttgta | 12120 |
| ttttttttt  | tttttttta | agaaagttt | gttttttt   | gaaatgatt  | ttttttgta | 12180 |
| gtatgtttt  | tttttaatt | gattatttt | tttttttt   | ttttttttt  | tttTGATAT | 12240 |
| AGTGAGTGA  | TTTGTAGAG | ACGGATAGT | AATATTTCG  | GGTGTAGTA  | TATTAATGT | 12300 |
| AATTATAITA | AGtttagtg | tgtggttao | gtgagagag  | aggttgggt  | ttgggaatt | 12360 |
| tttttttgt  | gagagagtt | taggattga | gataagttg  | taaaagcaa  | attgttttt | 12420 |
| tgtatggt   | aaaaggtag | tggttttag | ttttgtttg  | tataagagt  | tattgtttt | 12480 |
| gagatggt   | tttttaagt | tgatttgga | ATTAGTGT   | AGGTTTGA   | GAAGAAGTA | 12540 |
| AGATTATAT  | GTTAATTAG | GTTGTTTGG | AGGTACGAT  | TAAATGATT  | TGTAGATGG | 12600 |
| CGTGTAGGA  | GAATATGCT | GTATAGTTA | TGATTATGA  | AGAGGCGAG  | AAAGGTCAG | 12660 |
| ttagggagt  | ttttttttt | tatagtttt | togtttggt  | gattgtatg  | taagtgtga | 12720 |
| gagttagtg  | atgttaggt | agaaaggtt | tttaaggtt  | aggtttagt  | ttttgtttg | 12780 |
| aaatagagt  | ttagtgtaa | agtttagat | ataatagt   | atttttagt  | tttttatgt | 12840 |
| atttttggt  | tttttttgt | ttttgattt | tttttttagt | tgttttttt  | ttttttttt | 12900 |
| ttgttttgt  | tttttttgt | taggttttt | tgttttttt  | tttttttatg | atttttttg | 12960 |
| agtttttgt  | tttttttgt | ggttttttt | ataagtttt  | ttttgtgtg  | tttttaggt | 13020 |
| gatttttgt  | gtttataaa | tgggttatg | ttttttttt  | tttgaattt  | tttttttgt | 13080 |
| ttttgttgt  | ttttttgta | aagttagtt | ttaaagttt  | gttttaagt  | ttttgttgt | 13140 |
| tttttttgt  | tgttttgtt | tttttaagt | tatttagtt  | ttagtttgt  | tagtttttt | 13200 |
| aggttttgt  | tttttttgt | tataagttt | tttttttgt  | gaaatgatt  | tttttttat | 13260 |
| tttttttgt  | tgttttgtt | ttagttttt | aaagttagt  | cagagtttt  | tttttttgt | 13320 |
| gatttttgt  | ttgttttgt | tttagtttt | gaggaagat  | taagtgttt  | ttagttagt | 13380 |
| tttttaggt  | ggttttttt | togtttttt | tttttttgt  | tttttttag  | gtatagagt | 13440 |
| atagatttg  | gttttttgt | gttttagag | tgttttgtt  | gttatagtt  | agagattat | 13500 |
| tttttttgt  | gttaagttg | tttaattga | aaagaagta  | togtttagt  | ggttttagt | 13560 |
| gttttttgt  | tttttttgt | tttagtttt | agaaagttt  | aggttttgt  | tttttttgt | 13620 |
| agtttttgt  | tttttttgt | aaatgtttg | gtogtttta  | ggtatttag  | atatagagt | 13680 |
| tttttttgt  | tttttttgt | tttttttgt | tttttttgt  | tttttttgt  | tttttttgt | 13740 |
| atgAATGA   | TGCTTTTAT | ATTGTTTGA | GCTGGGTAT  | TATGCTGTT  | ATGGTTTTT | 13800 |
| TTTGTGATT  | AATTCGAGG | AGTTATGAT | AATCGAAT   | AAATTCGTA  | TTTATAGGT | 13860 |

[Drawing 34]

TTTTGGTG GATAATGta gtagtttta ggttttggt tatttggga gttttttt 13920  
 ggtttgtt tttttttg tgggtagg tgggtgggt ggtgttto gggagggg 13980  
 gggatattg tttttggt tgggttag GAGATTGAT TGGGAGATT TGTATTATT 14040  
 AGTATTGAG TTGTTGAT TATGGGTTT TTAGTGATT TGGGGTGT TTAGTTTTT 14100  
 TGGATTAGAT TAATTAGCG TAGGAAATT TGTTTACGT AGGTTTATT ATGGTGATT 14160  
 GTAGtgagg atgataatt tgggttagt agttaggtt gggagtaga tattgttaag 14220  
 tggtagagt tggtagagt atataaag tgggttaga tattgttgt tttgttta 14280  
 tttgttgtt tttgttgtt ttttttgtt tttagaatt tgggttaga gttgttaga 14340  
 ttgtttttt tttgttttt tttgttgtt tttttttt aggtttttt tgggttagt 14400  
 tattgttgt attttgtta atataagg aggttaggt gtttgttg ttgttaggt 14460  
 ttaggttag tgggttagg tgggttagt attttgtta gtttgtgt ttgttgttt 14520  
 gttttttt ggggtttta tttgttgtt tttttttt aggtttttt atggttagt 14580  
 tttttttt tttttttt ttttttgtt tgggttagg gtttgtgt ttttgtgtt 14640  
 tttttttt ttttgttt ttgttgtgt gttttttt gttttttt gtttgtgtt 14700  
 gtttgtgtt ttttttgt gtttgtgtt atttttttt tttttttt gtttgtgtt 14760  
 ttttgtgtt attttgtt aggttaggt gtttgtgtt tgggttagg tgggtttt 14820  
 atggttagt ttgttgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 14880  
 ttttgtgtt ttgttgtt gtttgtgtt atttttttt atttttttt ttttgtgtt 14940  
 ttttgtgtt attttgtt ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt 15000  
 gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 15060  
 ttttgtgtt attttgtt ttttgtgtt ttttgtgtt gtttgtgtt attttgtt 15120  
 ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt 15180  
 ttttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15240  
 ttttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt 15300  
 gtttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt 15360  
 gtttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 15420  
 ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15480  
 ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15540  
 ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15600  
 gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15660  
 gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15720  
 ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15780  
 gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 15840

# [Drawing 35]

ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt attttgtt 15900  
 ttttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt 15960  
 ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt 16020  
 GGTATGCGT GGTATGCGT TATTATTGT TATGATATG TTTATGAGA ATATTTTTAT 16080  
 TAAGGAGAG gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 16140  
 ttttgtgtt gtttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt 16200  
 TTAGAGATT ATTTAGATG TGGGGGCGT GGTTCGGGT ATGGTGAGA GGGAGCGTA 16260  
 GTATAAGTT ATTTAGCGT TTAGGTTTA GTTTATGAA ATTATTAAGA AGAGGTTGA 16320  
 GGTTTGTAG gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 16380  
 gtttgtgtt gtttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt atgttgtt 16440  
 AAGGTTAGG AGTCGAGTA GGGGAATATT ATTTATTTT TATTATGAA GAATGTTAT 16500  
 GTTAAGGTT TTGTTATTC GTTTAGTga gtttgtgtt ttttgtgtt ttttgtgtt 16560  
 ttttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 16620  
 gtttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 16680  
 gtttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 16740  
 ttttgtgtt gtttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 16800  
 gtttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 16860  
 atgttgtt atgttgtt ttttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 16920  
 TTTGTATAT AAGGATAGA GGGAGGAGA AGTGAAGA TAGCGTTAG TATATAAGA 16980  
 GAAGAGTAG GGTTTTTTA AGAGGAGTG AGCGGTGTT TTTTAGGTT GTTATGtat 17040  
 gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt atgttgtt 17100  
 ttttgtgtt gtttgtgtt ttttgtgtt ttttgtgtt ttttgtgtt gtttgtgtt 17160  
 gtttgtgtt gtttgtgtt ttttgtgtt attttgtt ttttgtgtt gtttgtgtt 17220  
 AGTTTGTAT TTTGTAGT ATTTGCGAT GGTATGATT ATATTTGAA TTTAGGAGT 17280  
 TTTTATTTT TTTATTTTA ATGTTGTA TTTTTTTT TTTTTTTA TTTGTATAT 17340  
 AGTTTGTAT GGTTTAGGT AGGTTAATT TTTTTTTT TTTTATTA GTTTTGTAT 17400  
 TATTgtgtt gtttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 17460  
 ttttgtgtt atgttgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 17520  
 gtttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 17580  
 ttttgtgtt ttttgtgtt gtttgtgtt ttttgtgtt gtttgtgtt gtttgtgtt 17640  
 gtttgtgtt gtttgtgtt gtttgtgtt attttgtt gtttgtgtt gtttgtgtt 17700  
 attttgtt ttttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt gtttgtgtt 17760  
 gtttgtgtt gtttgtgtt attttgtt attttgtt ttttgtgtt ttttgtgtt 17820

### [Drawing 36]

|  |       |
|--|-------|
| atggaatgt gaggtagga ttgaattta ggtgttga tttagagtt tatattttt     | 17880 |
| atttgaagt ttagttgag gttatttta ggggggttt gattatatt tttagagtt    | 17940 |
| gagttttaga ttgaattaa gaggatagt taatgttgg aaggtagat ttgagttag   | 18000 |
| ttogtttggg ttttttttag cgggaatag gataggttt tttagatatt cgggtaggt | 18060 |
| tagtttttt tttatkttag gtgtgtgtg tttttattt tttagstaga gtttagaag  | 18120 |
| ttggttagag tgaagagtgt ttgtttttt gtttttttt tttttttta ttttagaggt | 18180 |
| gggtttttt tttatttga atattgtgt tttagggag taagtoggt ggggtattt    | 18240 |
| tttttagaag tgttgogtt tatttttga aaggttaga tagtataggt tttaagttt  | 18300 |
| agaggttgg ogtatttat ttatgaatt ttatagagt tttagoggt ataggtattt   | 18360 |
| atagttttc gatttttgg aatttagag gtttagtag gaga                   | 18404 |

### [Drawing 37]

|   |      |
|---|------|
| ttttttgt tagtttta agtttagga agtagggga ttgtaatgt ttgtgaogtt    | 60   |
| ggggttttg taaggattg ttgaatag taogtttag tttaggitt taggattgt    | 120  |
| gtgttttag ttttttag agttgogtt agtattttt gaggatgat tttaaggt     | 180  |
| ttttttttt aggtattag ttttttaatt taagaagaa tttttttt gattggggg   | 240  |
| aaggagagg ggtataaaa taagtattt tttaogtag ttatgtttt aaatttgtt   | 300  |
| taaggagta aggttagtg cgttttgat gaggagga gttgagtg ttoggtgtt     | 360  |
| ttgagaggt tgttttgtt ttogtttag gggcatagt caattggtt tttagttga   | 420  |
| gtttoggtt gtaattatt tttttagtt tagatttga attaatatt agggaggt    | 480  |
| gattagagtt ttttagagg tggtttagt tttatttta ggttaggag ttttaggtt  | 540  |
| gggttaggt agtttagtt agattttag tttattgtt ttatagttt gtgttttag   | 600  |
| gtaagtatt aatttatta gtttttgtt ttttagttt ttgttaggt aaattgaga   | 660  |
| ttatagaggt gtttagatt aatttaggt tttagtttt tagttttg gtttaggtt   | 720  |
| aggttgtgt gatttttgt ttogtttta ttgttagtg ttattoggt gtttttata   | 780  |
| attatttgt taataggtt tttatttta aaggaggga ttggaggtt ttatttagg   | 840  |
| ttttttttt aogtgagga aggttttag atttagtto gtttaggga aggttaggt   | 900  |
| tttagogag gtagtagga aggtatatt agttattat ttgtttgt tttttgttg    | 960  |
| ttttatttt ggttagtaa aggttttag ggttatatt AGTGAATTA GGGTTTATT   | 1020 |
| TATAAGAGA GAAGGTTGG TTTTGTGG GGTTTGGTG GGTATATAT AGGTTAAGG    | 1080 |
| AGAGTGGG GGAATGAT TATTAAAT ATAAAGAAAT GGGTATTTT TAGGTTAAG     | 1140 |
| TTGTAGTTT GTTATGCG AATGTTTT ATAGGTTAG GGTGAAGTT gtttagaga     | 1200 |
| gggttagga gatttagga gatttagga tttagggtt gaattaggg tttagggtt   | 1260 |
| gttaggagt atagtagga gtttaggtt tttatttat tttaggagg ttgttaggt   | 1320 |
| aaggtatag gtttagtag ggaaggtatt taggttagg agtttatTA TGGTTATTG  | 1380 |
| AGGATAGTAT GGTATTAT TTTTGAAGG AATTTTGT TTTTTTTG TTGTTGATC     | 1440 |
| GTGTTTTT TATTTTTT TTTTGTGT TTTTAGTGT TAGGTTTTA TATATATTT      | 1500 |
| TTTTGTGTT gtagtggtt gtagggagt aagttaggt agtttggtt gtttagaga   | 1560 |
| tagtatagg gatttaggt ttgttagtt tttttggtt ttogtttta taggttaggt  | 1620 |
| agtttaggt ttgttagg attttaagtt tttagaggt aggttatata ttttatatac | 1680 |
| gagtggtt ttgttatata gtagggttt ataggttg gtttagga aatttatag     | 1740 |
| aatagagg gtaggatta agtttaggt tttagtagg ttttttagt tttaattatt   | 1800 |
| gttttttta ggaagtagg ataggttag gtaggtata ggttaggtt ttgttaggt   | 1860 |
| gtagtaggg ttatttatTT GAGGAGGTG GGGAGGTTT TGGTATGGG ATTTTATAG  | 1920 |
| GTGAGGAT AGGTATGTT TTGTATTTC GATTTTGGT TTTTTCGA tttaggttag    | 1980 |

### [Drawing 38]

aggentagtg ggaagtggt ggttttagtg aggttagtg gtttttttt tgtattgtag 2040  
 tttttttttt ttttaggttt tgttttagtg gttttttttt gattttttttt tttttttttt 2100  
 TGGTTTTTAAAT GAATTGGGCG ATGGTTACGT AGATGAATTT GTATTGGGTT TTGTTTGTGA 2160  
 TTATGTTGGA GGGTTGGGTT GGTATTATTT GAGTGGTTTT TTGGATTTA ATGTTATAGT 2220  
 TTAGGTTtag gtaggggtag gttaaatttt aggttttagt atagggggt atoggggggt 2280  
 tgtttatttt ttatttttaa atttttaggt gtttttttt TTGGTTGGAG ATGTTTTTGA 2340  
 TGAGTATGTC GATGATAATG ATGGTGTGTT TGGGTTGAT GTGGGCGttt ggaagagag 2400  
 ggggtgttat tagaggttag ttaagttttt ttgggggag ggggtattta ggttttagtt 2460  
 ttagttagtg gtttttagtg agtagtttg gtttttagtg ataggggga atgtgttag 2520  
 ggttttaggg taggttagtg aggttttg ttttttagtg tatgttagtg ttttttagtg 2580  
 ggttttaggg tgttttaggt ggttttagtg ttttttagtg tttttttttt tttttttttt 2640  
 ggttttaggt tttttttttt gtaggttagt gttttttttt ggggttagtt ggtttttttt 2700  
 attttttttt tttttttttt ggttttagtg ttttttaggt ttttttaggt ttttttagtg 2760  
 ggttttagtg aggttttttt agtttaggtt tttttttttt tttttttttt ttttttaggt 2820  
 ggggaagaga tagtttttag atagtaggtt ggggttaggt aggttttagg gtttttaggg 2880  
 agtagtagtg taggaaggtt tttttttttt ttagttagtg gtttttaggt tttagggga 2940  
 ttttaggttt atttttaggt ggttttagtg agtttaggt tttaggttag ttttttaggt 3000  
 tttaggttag atgttagga atattttttt atattttttt ttttttagtg acatttttag 3060  
 aaaggttagt ggttttttag ggttttagtg atgttaggt tttaggttag gtttttaggt 3120  
 agtttttagg ggttttagtg ttttttagtg agtttaggt aggttaggt tttaggttag 3180  
 tttaggttag gtttttagtg atgttagtg ttttttagtg tttaggttag atgttaggt 3240  
 ggggaagaga aggttagtg aggttaggt aggttaggt tttaggttag ggttttaggg 3300  
 ggttaggttag aggttaggt tttttttttt tttttttttt tttaggttag tttaggttag 3360  
 agtttaggt tttaggtttt ggttttagtg gtttttagtg tttaggttag aggttttag 3420  
 tttaggttag aggtttttt ggttttagtg ttttttaggt ggttttagtg aggttttag 3480  
 agtttagtg atgttagtg gtttttagtg tttaggttag ggttttagtg ggttttagtg 3540  
 ttttttaggt atgttagtg gtttttagtg tttaggttag ggttttagtg ggttttagtg 3600  
 tttaggttag ttttttaggt atgttagtg aggttttagg atgttagtg tttaggttag 3660  
 tttaggttag atgttaggt aggttttagg atgttaggt atgttaggt tttaggttag 3720  
 ggttaggttag aggttaggt tttaggttag ggttttagtg ggttttagtg ggttttagtg 3780  
 aggttttagg gtttaggttag ggttttagtg ggttttagtg ggttttaggt tttaggttag 3840  
 ttttaggttag aggttttagg aggttttagg tttaggttag ggttttagtg tttaggttag 3900  
 ggttttaggt aggttttagg tttaggttag tttaggttag tttaggttag tttaggttag 3960

# [Drawing 39]

ggttttaggt tttttttttt tttaggttag ggttttagtg ggttttagtg tttaggttag 4020  
 tttaggttag ggttttagtg ggttttagtg tttaggttag tttaggttag tttaggttag 4080  
 tttaggttag tttaggttag ggttttagtg ggttttagtg ggttttagtg ggttttagtg 4140  
 aggttaggttag tttaggttag tttaggttag tttaggttag tttaggttag tttaggttag 4200  
 tttaggttag tttaggttag tttaggttag tttaggttag tttaggttag tttaggttag 4260  
 TTGTGTTGA GATGATTTT TTGTGTTGA GTTGTGTTGA TTGTGTTGA TTGTGTTGA 4320  
 TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA 4380  
 TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA TTGTGTTGA 4440  
 tttaggttag tttaggttag tttaggttag tttaggttag tttaggttag tttaggttag 4500  
 aggttaggttag aggttaggttag tttaggttag TTGTGTTGA GATGATTTG AAGTACGGA 4560  
 GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA 4620  
 GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA 4680  
 ggttttaggt tttaggttag ggttttagtg tttaggttag gtttttaggt atgttttagg 4740  
 tgttttaggt aggttttagg atgttttagg tttaggttag ggttttaggt ggttttaggt 4800  
 atgttttagg tttaggttag tttaggttag tttaggttag ggttttaggt ggttttaggt 4860  
 tttaggttag ggttttaggt tttaggttag tttaggttag ggttttaggt ggttttaggt 4920  
 tttaggttag atgttttagg atgttttagg atgttttagg tttaggttag ggttttaggt 4980  
 ggttttaggt atgttttagg tttaggttag tttaggttag ggttttaggt tttaggttag 5040  
 ggttttaggt tttaggttag tttaggttag tttaggttag atgttttagg ggttttaggt 5100  
 tttaggttag tttaggttag ggttttaggt tttaggttag ggttttaggt ggttttaggt 5160  
 tttaggttag ggttttaggt tttaggttag ggttttaggt tttaggttag ggttttaggt 5220  
 tttaggttag tttaggttag ggttttaggt ggttttaggt ggttttaggt tttaggttag 5280  
 atgttttagg tttaggttag atgttttagg atgttttagg ggttttaggt ggttttaggt 5340  
 tttaggttag ggttttaggt tttaggttag tttaggttag ggttttaggt ggttttaggt 5400  
 ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt 5460  
 ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt 5520  
 ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt 5580  
 ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt 5640  
 ttttttaggt tttaggttag tttaggttag tttaggttag ggttttaggt ggttttaggt 5700  
 ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt ggttttaggt 5760  
 TTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA 5820  
 ATGTGTTGA GTTGTGTTGA ATGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA 5880  
 GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA GTTGTGTTGA 5940

# [Drawing 40]

|            |             |            |            |            |            |      |
|------------|-------------|------------|------------|------------|------------|------|
| tggtttaga  | tgggtttga   | attattgta  | tttttttta  | tgtaagaat  | tagtttaagt | 6000 |
| tttttttagt | tggttttga   | tttaggttt  | tttttttga  | taggaattt  | ttagggttta | 6060 |
| gttttttttt | tttaggtgt   | tatatgttg  | etTTTGATGT | AGTTGGTATT | GATGTAGTGA | 6120 |
| GAATGAGGGA | TGTTATTGTT  | TGTTTTTGT  | AGGATTATTG | GATTGTGATT | AATTggagat | 6180 |
| agagagagag | agagagagat  | ggtttgagt  | agtagaggt  | ttattatatt | tgggttagat | 6240 |
| ttttttagt  | taattaggtta | ttttgtttt  | agaggttatg | aggtttgagg | aggtttgttg | 6300 |
| gtatagggg  | tgagggatta  | tttgtatga  | agaggtgag  | agttttata  | agaggttgg  | 6360 |
| agtaggggt  | aattgtttg   | ttgtgtatg  | agaggtttt  | ttggagagag | tagaggtttt | 6420 |
| ttggagagag | ggaggtttt   | gtagggagt  | tgagagagt  | gtttttttg  | gagtggagag | 6480 |
| tgagaggtg  | tgaggtgag   | ggaggtagt  | ggaggtgag  | aggtttttg  | gagtggagag | 6540 |
| gtaggttgg  | gttttatAG   | GAAGAAATTT | TTTGTAGCG  | TTTTTGTITT | TGTTTTTGG  | 6600 |
| TGTTTGTITT | TTTAAAGGTT  | GGTGAAGTT  | TTTATTTTIT | TGTTTTTGT  | AATTttgaga | 6660 |
| tttaggtgg  | gaattgagta  | ttagttttt  | agaggttat  | ttatggagag | agtttaagt  | 6720 |
| aggtgggtta | aggttagag   | tgagtagga  | ggagaggtg  | ttgttttgg  | gitttaggt  | 6780 |
| gttgtatata | tttaggtgt   | tttagtttg  | tggttttta  | ttatgtatTT | TAAATTTTT  | 6840 |
| TTAGAAAGTA | GTTTGGTTG   | TATTTTGGG  | TTTTTGTITT | TTGTTAGTT  | TTAATATTGA | 6900 |
| GTTTTAAATG | TTAGTGTAT   | TTATTTTGT  | GGTATAGTAC | Ggttgaggtg | ggagagagag | 6960 |
| agaggttagt | aatttagaga  | tttagagat  | ttatatttg  | ggatagttt  | agagagagtg | 7020 |
| ggaggttagt | tggttttatt  | tttagTTGT  | GGTAGGTAGA | TAAAGGCGTT | TGAGGTTTTT | 7080 |
| TTAATTTTGA | TTTTTTTGA   | ATTTTTTAT  | AGTTTGTGA  | GTTTGTGAA  | GGTTTTTAAA | 7140 |
| TTATTTATG  | TGTAGCGTTT  | ATTTggag   | gagttatatt | aggttttta  | taggatttga | 7200 |
| gttttttttt | ttgggttttt  | agtttgagt  | tttaggttg  | gtagttttt  | ttgtttatt  | 7260 |
| atattgtgt  | tatagtagg   | agtttagta  | gaggttttt  | ggtgttttg  | ttgtttatt  | 7320 |
| TGATATATGA | TTTTGATGT   | GGTATTTTG  | AGCGGGGAGT | TTGGTTAGT  | TTTGGTTGG  | 7380 |
| TTATTAGATA | TAGAAATAG   | GAAGTTTTTA | GGTTGGTTGA | GTTTTTTAG  | TATAGAAAC  | 7440 |
| GTTTAGGTT  | CGTTTTTGT   | TTGTAGTAT  | GTTTTGTITT | GTTGTTAGA  | TATGTGGTTA | 7500 |
| TGGTATTAT  | tagagaggt   | aggttagga  | aggttagat  | ttagttata  | ggagagagag | 7560 |
| ttgagtagag | atatttatag  | agagagtag  | gaatttagt  | atttatgtg  | tgtaggtat  | 7620 |
| tgagttatat | gtttttaaat  | ttatttttt  | atttaggtt  | tttatata   | tgtagagag  | 7680 |
| taggttatgt | atatttttta  | tttttagat  | ggagaggtg  | aggttaggt  | gtttttatt  | 7740 |
| agtttaggt  | tttttagga   | tttttagga  | agtttttag  | gtttgttg   | ttaggttgg  | 7800 |
| gttagtagg  | ggaggttag   | tttttttag  | tttaggttt  | ttaggttta  | gttttttag  | 7860 |
| tttttaggt  | tttttagg    | tttaggttt  | aggtttatt  | tttagaggt  | agagaggttg | 7920 |

# [Drawing 41]

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| staattatta | aatattigg  | agagagaga  | ttagagtga  | atttttgtga | agattaggt  | 7930 |
| tgtgttttag | ttagattgtt | agrtanagag | naagttagat | agaggttata | ttataggag  | 8040 |
| tgtgtggaga | tggatata   | tttttagtt  | ataaggag   | agaggttgtt | gagtttgtt  | 8100 |
| agagagagag | agaggaatat | tatttttgg  | aaagagtgat | ttagaggtgt | tttgttgtt  | 8160 |
| ttggtatgt  | ttattggag  | ggagtttag  | gtttanaag  | atttagagga | gttgttagg  | 8220 |
| tagaagaat  | aatttagtt  | ttttttoga  | ttatatttt  | tttttagaga | gaatttttag | 8280 |
| ttttataag  | ttattgttt  | taggaaatta | agtagatga  | aaataataa  | atttttttt  | 8340 |
| ggagagagt  | gagagtgag  | gttgttagt  | ttttttagt  | taggagaga  | ggagagaga  | 8400 |
| gtaaatttt  | taattgttag | gtaggggga  | agggagagag | gagattgtt  | ttttaatttt | 8460 |
| ttttttttt  | gtttagagat | gatttttag  | gagagattt  | ttagattttt | taatttttt  | 8520 |
| aggtatttt  | gttttaggt  | tttggttag  | gttagagtt  | ttagtaggg  | ggaggggtt  | 8580 |
| gagtttttt  | tttttagtt  | taggatgag  | gaatttttt  | gtaaagtta  | ggttgattat | 8640 |
| aggaagatg  | gtttatoga  | aggtatagt  | taattttgt  | tagaagaga  | aggtataga  | 8700 |
| taaggtaga  | gattatag   | aatttgagg  | aaagagttt  | ttatatttt  | tttagtttag | 8760 |
| agtagatgt  | gttttttga  | agaggttat  | taaaaggtt  | tttagtggg  | tttttttgt  | 8820 |
| tttaatttt  | ggttttoga  | tgttttttt  | ttttttttt  | ttttttttt  | ttttttttt  | 8880 |
| tttttagttt | tgttttttt  | tgtgggggt  | tttttaggt  | ttgtttttt  | ttttttttt  | 8940 |
| tagtattg   | ttagtttgt  | ttttttttt  | ggttagagt  | aaagagaag  | agagatagt  | 9000 |
| agtttttag  | ggttttggg  | ttaaaagtt  | tttgaggt   | ggagtgaga  | agaggaaga  | 9060 |
| tagggttag  | ataagggtt  | agttataga  | ttttataga  | gagaggaag  | gtttttaga  | 9120 |
| aggtogatt  | aggaagaag  | gttttttga  | tgtttatag  | ggttgtagt  | tgtagaatt  | 9180 |
| gtattttag  | tttaattag  | tggtttgtt  | aaataattg  | atgtatttg  | gagttoga   | 9240 |
| tttttagaga | gttgtaggt  | gtaggggta  | aggttttga  | ttattttta  | aatogatttt | 9300 |
| tgaagttag  | tttttaaggt | aaaggttagt | aggttttgt  | taggagta   | tgttaattt  | 9360 |
| ttttaaggt  | atttttgtt  | ttttttttt  | taaggtttt  | ggtattaga  | tgtgttgtt  | 9420 |
| gaagagatt  | attgttgtt  | ggagagatt  | ttaggtttt  | agtttttgt  | ttatgtttt  | 9480 |
| gaagattat  | agagagagt  | gggggagta  | taggagaga  | atgttttag  | ttatttttg  | 9540 |
| tgtttaggt  | aggttagat  | atttttata  | gttaaatat  | tttatagt   | tttttaaga  | 9600 |
| tttttttgt  | gttgagagt  | ttatttgtt  | ttatagata  | gattttatt  | tacagagag  | 9660 |
| tggagatag  | gtattagt   | ttttggtag  | tattttttt  | ttttttttt  | tttgtttag  | 9720 |
| tttttttgt  | ttttaattg  | tggttttgg  | agaggttag  | ggaggaagt  | aggttttgt  | 9780 |
| tttagagag  | aaagattgt  | taattataga | gttgttgt   | agatattgt  | gttttaggt  | 9840 |
| atgtttttt  | tttttttag  | tgggtttgg  | gttgttagg  | tttagtttt  | aggttttagg | 9900 |

[Drawing 42]

|            |            |             |             |             |             |       |
|------------|------------|-------------|-------------|-------------|-------------|-------|
| agagtaggtt | tttttgattt | tttttggggt  | taggtagaaa  | gggttagaga  | ttaggttttg  | 9980  |
| agatattagg | tttagagata | gtagagagga  | gggtatttga  | gggttttttg  | tatgtataga  | 10020 |
| agggagaggg | atggaggggt | ggatgttggt  | gtttgttatt  | tttttttttt  | tttttttttt  | 10080 |
| tagggtggga | ttgtttttat | tttttttttg  | gagtaggggg  | gagtaggggt  | tttttttttt  | 10140 |
| TATTAGTAGG | ATGAGTAGAT | TTTAGGAGGT  | ATTTAGAGGT  | GATGATGTGT  | TGTGTAGAGT  | 10200 |
| TTTGTAGAGT | ATTTTGTGTG | TGAGTGTAGT  | ATTATTATAG  | TTTGTGTAGA  | GTGTGAAAT   | 10260 |
| TTTTTTTGT  | ATATAGTTTA | TAGAAATTTT  | TTGAGTTTGT  | TGTGTGAATA  | TGGGTTATTT  | 10320 |
| GATTTTTTAT | Ttttagaggt | attagggoggt | ggagtttagc  | gggttagggg  | tagggtaggg  | 10380 |
| aggtatagga | tcagtttagg | ttttgggttg  | gggttatatat | gggttatagtt | gggttatagtt | 10440 |
| agggataggg | atgttagggg | ttaggggttg  | gggttatagat | ggggtagttg  | tattgttagt  | 10500 |
| agggatttag | aggttaggtt | ttaggggttg  | gggttatagat | ggggtaggtt  | aaattgttag  | 10560 |
| aggttagggg | aggtttattt | attTGAGGGA  | GAGGAGAGAG  | TTATTTTGAT  | TTTTGCGAAT  | 10620 |
| GGGTTGAGTT | AGGAAGTAT  | GGTGATATT   | TGCGTTTTTG  | AGTAGAGTTT  | TGTATTATAG  | 10680 |
| TTTTATTGAG | TTTGGGTGAA | ATATTTggg   | gggttttagt  | aggttagtga  | ggaggggttt  | 10740 |
| ctgtgatttt | aggttttttt | ctgttttttt  | tatttaggttt | ctgtgttttt  | tatttttttt  | 10800 |
| TTTTGGGGGT | TTTTGGGAGG | GAGGAGGGGG  | TAGGGAATGA  | GGAAGTTGTAG | TTAGTTTGGG  | 10860 |
| TAGGTAGAGA | GTTTAGGGAT | TAGGTTTTAG  | ATTGATTTTT  | TAGTTGTGGG  | TGGTTTTATT  | 10920 |
| ATTTTGGGTT | TTTTGGGTTT | TGAGGTTTAT  | TTTTATTATg  | ttttaggttag | ttaggttagg  | 10980 |
| ggggataggg | gggggggggg | tcagtttttt  | gggggaattt  | atttgttttt  | tttgtttttg  | 11040 |
| agtatagtag | gggagagaga | gttagatagg  | gttagatttt  | gggtggggag  | gggttatatt  | 11100 |
| ttttataggt | ttagtataga | aggttttagg  | gttagatttt  | gttatatttt  | gttatatttt  | 11160 |
| gttagaagga | tatttatgtg | tttgttatat  | tttaggtttt  | gatactattt  | tacgtataga  | 11220 |
| aattttagag | gttttagogt | attttttttt  | tttttttagg  | ttgtttttta  | gtagtttttt  | 11280 |
| tgtatttagt | tcgttataga | tgatgtoggt  | gatatgtttt  | atogagttag  | tttgttttag  | 11340 |
| tgtagggggt | tgttttataa | tgaggtttat  | tgatttttgt  | tgatttttgt  | tttgttttag  | 11400 |
| ttttatagaa | tagggttat  | tttttatatt  | tatatagata  | ggattttggg  | gttttagggg  | 11460 |
| tttaggttat | tggtttgatt | tattgttagt  | tttgttttag  | tgatttttag  | taatttttag  | 11520 |
| tagttaggtt | ttaggttagt | tggttttagt  | ttttttatat  | tgatttttag  | tttttttagt  | 11580 |
| aatagagatt | aaaggttttt | ttgttttaag  | gggtttttat  | gggatttagg  | gatttttagt  | 11640 |
| taagattttg | tttaggtttg | taagagagaa  | gggttttttt  | ttttttttta  | tattgttagt  | 11700 |
| ttgttttagt | tattgttagt | gaggttagga  | taatttttag  | tttaggtttt  | ttgttttagt  | 11760 |
| gggttttttt | taatttttag | ttttgttagt  | ttgtttttgt  | tttagttttt  | tttagggggg  | 11820 |
| tttttttttt | tttttttttt | ggataggttt  | tttttttagg  | ttgttttttt  | tagggttttt  | 11880 |

# [Drawing 43]

|   |       |
|---|-------|
| tgattttaga gtttttaaat attaaggtat atgatttgt ttatttgaat ttttaaatat  | 11940 |
| aatatttaat atgtttaaat taanaogaga aaagttagtt taataggaga tatataagan | 12000 |
| atatatattg atgtttttt ttaggagaga aaattgaatg gttgagat aggttazag     | 12060 |
| gagatatttg gttttttata tattttttg aatgttatat gatgatatg tatattttt    | 12120 |
| ataaatata atagttaat tgggaataat ttttggaaa ttttaggt ataggagag       | 12180 |
| tttttggtt ttatttgat gttgttgtt taagtgtga taanaogtaa gttgtogag      | 12240 |
| ogtaggttt taogttgta atttagtat ttggaggt taagttggo ggttaogag        | 12300 |
| gttagaggt ogagatttt ttgttaata tggtaaat tttttttat taanaata         | 12360 |
| aaaaattag ttggtatg tggtaggtt ttgttaatt agttattog gaggttag         | 12420 |
| taggagatt attgaatt aggaatoga gtttagag agttaagatt atattattg        | 12480 |
| atttagttt gtttaatat taagatttta ttttaaaaa aaaaaaaaa taagttag       | 12540 |
| ttgtgtttt aaatttatg aaatttatg atgtttgt ttggtaaaa gaagaaag         | 12600 |
| ttttaagtt aatgaattt attttttat gtttaattt ttattttt tagaaatt         | 12660 |
| aatattaat gttttttt aaaaaaggt tttttttt tttttttt tttttttt           | 12720 |
| attgttttt gttttttt ttttttgt gttttgga attgtttg taattttt            | 12780 |
| gtatgttta gttttgta ttttttgt ttgttaata ttttagatt taggaatto         | 12840 |
| gagatttt taogtttao gtttaaggt aggaagata tatgaagt ggtttgag          | 12900 |
| gagattatt ogttgggt ttogttatt tggaggtt tagagttag tggatttt          | 12960 |
| ggtattggt aggtttta taataagtt ogttggtt tagttata ttgtttata          | 13020 |
| agtatttt ttgtttta tatattagt ttgtttta taagttagt aggtttata          | 13080 |
| tatattata gtatatat tttattatt atgtttatt atatatat ttgttgg           | 13140 |
| gtattttt agtatgaa tataaattg atttatag agttttag ttatttta            | 13200 |
| gtattaaag ttattgtat ttgtttta agttatttt tttaggttt aaattttt         | 13260 |
| agttggaag aattattog gtatttaga gaatttaatt aggttttt ttgtttta        | 13320 |
| tggagaggt aggaatttt ttgaaggt taagttag agtatagtt taatanaag         | 13380 |
| gtatgttt ttattttt ttgttagaa ataaaaatt atttttta ttgtttat           | 13440 |
| gtatgggt tttagattt ttttatat agattttt ttagtttta gatatttt           | 13500 |
| tgattagga attattttt ttatttttt atggaggaa ataaogtag ggaattag        | 13560 |
| aattataga gtaattgtt gttatttag aaaaaaaa tttttttt gaattagaa         | 13620 |
| gataaagta gatttaag ttaggtag ttgttaagt tttaattt agttttgt           | 13680 |
| gaggttag ttgtgatt atttatatt aattttag gtatttag aaatttag            | 13740 |
| aaatttgt ttttaaaa atataaatt tagtttag ttgtttata ttttttag           | 13800 |
| tttagttt taggaatt agttgga atgtttga ttggttag taagattt              | 13860 |

# [Drawing 44]

agttagttat ggttggtta tigtatctag gtttagtga tagatgaga ttttggtta 13920  
 aaaaataaaa atagatgga gtagatggt tttatattgt aatttagta ttttaggag 13980  
 tagagtagg aggtatttt gtagtagga gtttagatt agttggtaa tatagtgaa 14040  
 ttttattttt atagattgt aatttagtt ttttagggg tggatgtgt agtagtga 14100  
 gattatgta tttatttta gtttaggtaa tagatgaaa ttttggtta aaaaaaaa 14160  
 aaaaaaat tatatagatt agattatg gtagatag agatagga gtttaggag 14220  
 gtaaatagg taatatatt ttagaaggt ttttagatt ttttagatt tagatttto 14280  
 gtttagtaa ttagttatt aatttttt aattattgt gtagaagtt gtagatgt 14340  
 aggttttag ttttagatt ttttatata ttttatatt ttttaggta aatttatata 14400  
 gtaggtgta ttttatatt ttttagaata gtagataga ttttagatt ttagatt 14460  
 gtttagatt atagattat gtagtagg gtaggtaga taoggtgtt agtagtga 14520  
 gtttagatt tttttttt aatttatat ttagtagat agtaggag gtagatga 14580  
 tggattgtt tatatttat gtagaggt gtaggtaga gttttttt agtaggata 14640  
 tggattgag taagaggt gtagatag agtttatat gtaggttt gtaggtgt 14700  
 agtttatgt gtaggtaa agtttttt agtaggag aattttgt agtaggtat 14760  
 ttaggttag gtaggaat taaggtga attttatta gtaggttt gtaggta 14820  
 ttttagga aattattat agtaggtt aggttaga agtttaga gtagttat 14880  
 agtaggta gtaggta agtaggtt ttaggtag gtaggta gtaggtag 14940  
 taaggttag taggttag gtaggtta ttttatatt agttatt ttaggtat 15000  
 gtttatatt tttttttt tttttttt tttttttt aggttttt ttttatatt 15060  
 taggttag gtaggtag taatttag ttttatatt tttttttt gtaggttt 15120  
 taattttt gtttagatt ttttagatt ttaggttat agtaggtat tatgtgta 15180  
 gtttatatt tttttttt ttttagga gtaggttt tatattgt gtaggttag 15240  
 ttttatatt tttttttt gtaggttt ttttaggt ttaggttt gtaggttag 15300  
 gtttagga tatgttag ttttagga tttttttt tttttttt ttttagga 15360  
 tttttttt tttttttt tttttttt tttttttt tttttttt tttttttt 15420  
 tttttttt tttttttt tttttttt tttttttt tttttttt tttttttt 15480  
 tttttttt tttttttt tttttttt tttttttt tttttttt tttttttt 15540  
 tttttttt atagattt gtttatatt ttaggttag gtaggttt atatttat 15600  
 tatgttag gtaggttt ttaggttag gtaggttt gtttagtt ttaggttt 15660  
 taggttag gtaggttt atatttat gtttatatt gtaggta gtaggttag 15720  
 taggttag gtaggttt tttttttt tttttttt gtttaggt ttttaggt 15780  
 aggtattt tttttttt ttttttat ttttaggt atatttat ttttatata 15840

# [Drawing 45]

tatatttat tttttttt tagatgag gtttttat gtttagga ttttagga 15900  
 atttttag ttaggtatt tttttttt gttttttt agtttagga ttaggtatt 15960  
 agtttatatt atttttag gtttttat ttttatatt agtttat ttttatatt 16020  
 ttttaggt tttttttt ttttataga ttttttta agtttat tttttttt 16080  
 ttttaggt ttttataga ttttaggt atattttt ttttagga attttaggt 16140  
 ttttaggt agtttatatt gttttttt tttttttt atttttag ttttatatt 16200  
 gtttatatt agtttatatt ttttaggt ttttaggt taatttat ttttatatt 16260  
 gtttatatt agtttatatt gttttttt tttttttt tttttttt ttttatatt 16320  
 gtttatatt gtttagga ttttagga gtaggttat tttttttt tttttttt ttttatatt 16380  
 atttttag ttttagga ttttttat tttttttt atttttag ttttagga 16440  
 ttttatatt ttttagga atttttat ttttagga atttttat ttttagga 16500  
 ttttaggt ttttatatt ttttagga taattttt gtttaggt ttttatatt 16560  
 ttttaggt gtttaggt atttttat gtttaggt ttttatatt gtttaggt 16620  
 ttttaggt gtttaggt taatttat gtttaggt ttttaggt ttttaggt 16680  
 gtttaggt ttttagga gtttagga gtttagga gtttagga gtttagga 16740  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 16800  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 16860  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 16920  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 16980  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17040  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17100  
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 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17220  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17280  
 GGGGAGGG GGGGAGGG GGGGAGGG GGGGAGGG GGGGAGGG GGGGAGGG 17340  
 TTAGGAGG AAGGAGG TTAGGAGG TTAGGAGG TTAGGAGG TTAGGAGG 17400  
 TTAGGAGG AAGGAGG TTAGGAGG TTAGGAGG TTAGGAGG TTAGGAGG 17460  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17520  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17580  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17640  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17700  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17760  
 gtttaggt gtttagga gtttagga gtttagga gtttagga gtttagga 17820



# [Drawing 46]

```

gggggggat tttagttag gttaggttt gggtaggat taggttagt taggttata 17880
ggggggggtt gtttagggg atgggtttg gagggttag gggattagg gaggagaga 17940
tggggttgt taggttagt agagattgat atttagtta agttatagg gtatttatta 18000
ttgttggaa tattaaggtt ttttttttt ttttagtgg ataggggagg gttattttt 18060
aggagggagt taagttagt ttagtagtag gtttagggg tggtagggt atgggggta 18120
ggatagagt ttttagttg tttagtagt taggatttt agatitttg gtttagtag 18180
atttagtaa gaggtaaat ttaggtatg tttagaggg agtagggag ttaattttt 18240
tttagagat taggtgttt agatatttg gaggggtag tagtttagt atgatttgt 18300
ttttatttg ttggtagtt gttgttaa taggaagtat gtaggtttt gtttagtga 18360
gttaggaga tgaatttgg tttatattg gtatattag atgg 18404

```

# [Drawing 47]

(a)  
 Wild type DNA  
 5'-AGTCGCGATGCCAGCTCGCTCG-3' sense strand  
 3'-TCGAGCGCTACGGTCGAGCGAGC-5' antisense strand

(b)  
 Blauflited  
 5'-AGTCGCGATGTTAGTTGTTTCG-3' sense strand  
 3'-TTGAGCGCTATGGTTGAGCGAGC-5' antisense strand

(c)  
 FW primer 5'-AGTCGCGA  
 5'-AGTCGCGATGTTAGTTGTTTCG-3' sense strand  
 3'-TCAAGCAAGC-5' RV primer

(d)  
 FW primer 5'-AACTGCGA  
 3'-TTGAGCGCTATGGTTGAGCGAGC-5' antisense strand  
 3'-TTGAGCGAGC-5' RV primer

# [Drawing 48]

(a)

REP-S1 : 5' -CAGGCCAGTGGAGTGGCAG-3'

(b)

REP-AS1 : 5' -GAGGAGGTGCAGCTAGTCTG-3'

(c)

(#7441)

CAGGCCAGTGGAGTGGCAGGCCAGAACTGGGACCACCGGGTGGTGA

REP-S1

HpaII

GGCGGCCCGCACTGGGAGCTGCATCTGAGGCTTAGTCCCTGAGCTCTCT

HpaII

GCCTGCCAGACTAGCTGCACCTCCTC (#7500)

REP-AS1

### [Drawing 49]

(a)

REP-S2 : 5' -CAAAAGCACTGGCTTTGGAACC-3'

(b)

REP-AS2 : 5' -ATCGAGTGAGTCCTGCTGGAT-3'

(c)

(#0850)

CAAAAGCACTGGCTTTGGAACCGACTGTCTGGGTTTGAATCCTGGCACTG

REP-S2

Hpa II

CAGCTGACTCACTGATGGACTCAGGCAATGCCTTAACTCCCTGAGCCTC

AGGTTCTTGTCTGTAAATGATAAAGATAGCCCTGTTTCATAGGGCTGT

GGTGAGAAACCAATCAGACAAGGCATGTGAACGCCATTATAGCACAGCG

CCCGGCATCCAGCAGGACTCACTCGAT

(#7004)

Hpa II

REP-AS2

### [Drawing 50]

(a)

SHP1-PF1: 5' -TGTCTGGAGGCCACGGTCAATGA-3'

(b)

SHP1-PR1: 5' -GTTTGTATTCGGTTGTGTGATGCTC-3'

[Drawing 51]

(a)

SHP-LF1: 5'-GCCAGTTCATTGAAACCACT-3'

(b)

SHP-LR1: 5'-CCTTGTCTTGTCTTGTCT-3'

[Drawing 52]

(a)

MF2: 5'-GAACGTTATTATAGTATAGCGTTC-3'

(b)

MR2: 5'-TCACGCATACGAACCCAAAG-3'

(c)

(#7037)

GAACGCCATTATAGCAGCGCCCGGCATCCAGCAGGACTCACTCGAT

MF2

GACAGTTGTACCGCCATCATTGTTATTAGCGTGGGCCAGGGAGGCT

GCSTAAAAGCAGCTGGTGGAGGAGGGAGAGATGCCGTGGGACCGCTCT

GGGTTGGCATGCGTGA (#7195)

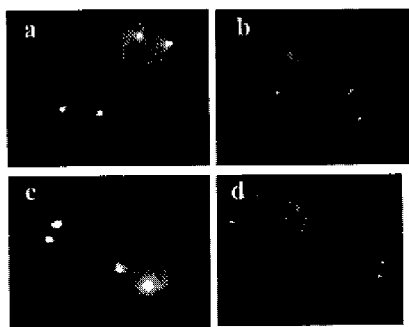
MR2

# [Drawing 53]

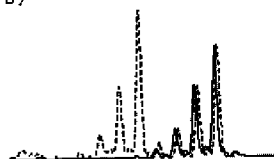
(a)

**FISH analysis of ILTMot and NK-YS cells with chromosome 12 or *SHP1* -specific probes**

| cells  | probe  | positive signal No.(%) |   |    |   |   |      |
|--------|--------|------------------------|---|----|---|---|------|
|        |        | 0                      | 1 | 2  | 3 | 4 | more |
| ILTMot | Ch #12 | 1                      | 1 | 97 | 1 | 0 | 0    |
|        | SHP1   | 1                      | 2 | 95 | 1 | 1 | 0    |
| NK-YS  | Ch #12 | 0                      | 0 | 99 | 1 | 0 | 0    |
|        | SHP1   | 1                      | 3 | 91 | 4 | 1 | 0    |



(b)



| <i>Microsatellite marker</i> | <i>LOH</i>   |
|------------------------------|--------------|
| D12S356                      | 15/19 (79 %) |
| D12S336                      | 6/16 (38 %)  |

## CORRECTION OR AMENDMENT

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[Section classification] The 1st classification of the part I gate

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C12Q 1/42

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G01N 27/447

G01N 33/53

G01N 33/566

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C12Q 1/68 ZNA A

C12Q 1/02

C12Q 1/42

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G01N 33/53 M

G01N 33/566

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[Filing date]June 3, Heisei 16 (2004.6.3)

[Amendment 1]

[Document to be Amended]Specification

[Item(s) to be Amended]0046

[Method of Amendment]Change

[The contents of amendment]

[0046]

Therefore, in this invention, SHP1 gene expression can be judged using four-fold marker by the maximum called loss of ornamentation and mRNA of gene DNA, protein, and allele. namely,

one hematopoietic organ tumor cell called a SHP1 gene-expression fall -- since a specific phenomenon can be checked in four steps, hematopoietic organ tumor cells are detectable by very high singularity.

(19) 日本国特許庁(JP)

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(11) 特許出願公開番号

特開2004-128

(P2004-128A)

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| (32) 優先日     | 平成14年4月5日 (2002.4.5)         | (74) 代理人 | 100080034                                   |
| (33) 優先権主張国  | 日本国 (JP)                     |          | 弁理士 原 謙三                                    |
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|              |                              |          | 岡山県岡山市津島中1丁目4番2-304号                        |
|              |                              | (72) 発明者 | 大内田 守                                       |
|              |                              |          | 岡山県岡山市門田屋敷2丁目2番58-106号                      |
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|              |                              | 最終頁に続く   |   |

(54) 【発明の名称】 造血器腫瘍細胞検出方法および造血器腫瘍細胞検出キット

## (57) 【要約】

【課題】 分子生物学的知見を利用して、造血器腫瘍細胞の高感度かつ高特異的に検出する造血器腫瘍細胞検出方法および検出キットを提供する。

【解決手段】 造血器細胞を含む検体試料中に含まれる、造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質を定量するとともに、上記検体試料から得られる S H P 1 遺伝子の塩基配列中に含まれる C p G 島のメチル化を確認する。これによって、一つの遺伝情報により造血器腫瘍細胞の有無を2段階で確認するため、非常に高い特異性で造血器腫瘍細胞を検出することができる。

【選択図】 なし

## 【特許請求の範囲】

## 【請求項1】

(1) 造血器細胞を含む検体試料中に含まれる、造血器細胞に特異的なプロテインチロシンホスファターゼSHP1遺伝子の塩基配列中に含まれるCpG島のメチル化を確認するSHP1遺伝子メチル化確認工程、

(2) 上記検体試料から得られるSHP1蛋白質およびSHP1mRNAの少なくとも一方の発現量を定量するSHP1遺伝子産物定量工程、および、

(3) 上記検体試料に含まれるSHP1遺伝子の異型接合性喪失(LOH)の有無を確認するSHP1遺伝子LOH確認工程、

の少なくとも何れかを含むことを特徴とする造血器腫瘍細胞検出方法。

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## 【請求項2】

上記SHP1遺伝子メチル化確認工程には、

上記検体試料から得られた遺伝子試料を、シトシンを含む塩基配列を認識するメチル化感受性制限酵素で処理する遺伝子切断試行段階と、

上記メチル化感受性制限酵素で処理された遺伝子に対して、上記SHP1遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識切断される塩基配列を含む領域を増幅するプライマーを用いて、ポリメラーゼ連鎖反応法(PCR)を実施する遺伝子増幅試行段階と、

増幅された特定のサイズの遺伝子の量を確認する遺伝子増幅量確認段階とが含まれることを特徴とする請求項1に記載の造血器腫瘍細胞検出方法。

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## 【請求項3】

上記プライマーが、さらに、配列番号1または2に示す塩基配列に含まれる部分塩基配列、またはこの部分塩基配列と相補性を有するポリヌクレオチドであることを特徴とする請求項2に記載の造血器腫瘍細胞検出方法。

## 【請求項4】

上記遺伝子増幅量確認段階では、電気泳動法を用いて特定サイズの遺伝子の量を確認することを特徴とする請求項2または3に記載の造血器腫瘍細胞検出方法。

## 【請求項5】

上記遺伝子切断試行段階では、制限酵素として、メチル化感受性制限酵素を用いることを特徴とする請求項2、3または4に記載の造血器腫瘍細胞検出方法。

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## 【請求項6】

上記SHP1遺伝子メチル化確認工程には、

上記検体試料から得られる遺伝子試料を、重亜硫酸塩で処理する遺伝子修飾段階と、

重亜硫酸塩で処理された遺伝子試料に含まれる、SHP1遺伝子の塩基配列中のメチル化シトシンの有無を判定するメチル化シトシン含有判定段階とが含まれることを特徴とする請求項1に記載の造血器腫瘍細胞検出方法。

## 【請求項7】

上記メチル化シトシン含有判定段階では、PCRによりメチル化シトシンを検出する方法、遺伝子の塩基配列の決定によりメチル化シトシンを検出する方法、またはメチル化シトシンを含む塩基配列を識別する方法のうち、少なくとも何れかが用いられることを特徴とする請求項6に記載の造血器腫瘍細胞検出方法。

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## 【請求項8】

上記遺伝子修飾段階では、重亜硫酸塩として、重亜硫酸ナトリウムが用いられることを特徴とする請求項6または7に記載の造血器腫瘍細胞検出方法。

## 【請求項9】

上記SHP1遺伝子産物定量工程では、SHP1蛋白質を抗原とするSHP1抗体を用いてSHP1蛋白質を定量することを特徴とする請求項1ないし8の何れか1項に記載の造血器腫瘍細胞検出方法。

## 【請求項10】

上記SHP1遺伝子産物定量工程では、酵素抗体法またはウエスタンブロッティング法に

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より S H P 1 蛋白質が定量されることを特徴とする請求項 9 に記載の造血器腫瘍細胞検出方法。

【請求項 1 1】

上記 S H P 1 遺伝子産物定量工程では、配列番号 3 に示す S H P 1 遺伝子 c D N A の塩基配列の全長またはその一部を検出するポリヌクレオチドを用いて S H P 1 遺伝子の m R N A の発現を検出することにより、S H P 1 m R N A を定量することを特徴とする請求項 1 ないし 8 の何れか 1 項に記載の造血器腫瘍細胞検出方法。

【請求項 1 2】

上記 S H P 1 遺伝子産物定量工程では、ノーザンブロッティング法、逆転写 P C R 法、リアルタイム P C R 法、または R N A i n s i t u ハイブリダイゼーション法により S H P 1 遺伝子の m R N A の発現が検出されることを特徴とする請求項 1 1 に記載の造血器腫瘍細胞検出方法。 10

【請求項 1 3】

異型接合性喪失の有無の確認は、上記 S H P 1 遺伝子を挟み込む 2 つのマイクロサテライト・マーカの少なくとも一方、または、上記 S H P 遺伝子中か、その近辺に存在する単一塩基多型のような遺伝子多型を、P C R を用いたフラグメント解析により実施されることを特徴とする請求項 1 ないし 1 2 の何れか 1 項に記載の造血器腫瘍細胞検出方法。

【請求項 1 4】

造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、

( 1 ) 造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質を抗原とする S H P 1 抗体、および 20

( 2 ) シトシンを含む塩基配列を認識するメチル化感受性制限酵素と、S H P 1 遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識される塩基配列を含む領域を増幅する P C R 用のプライマーと、上記 S H P 1 遺伝子のメチル化陽性及びメチル化陰性対照 D N A とのうち、少なくとも一方を含むことを特徴とする造血器腫瘍細胞検出キット。

【請求項 1 5】

造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、

( 1 ) 造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質を抗原とする S H P 1 抗体、 30

( 2 ) 遺伝子処理レベルまで精製された重亜硫酸塩と、該重亜硫酸塩で処理された遺伝子試料に含まれる S H P 1 遺伝子の塩基配列中のシトシンの有無の判定用プライマー、および

( 3 ) 配列番号 3 に示す S H P 1 遺伝子 c D N A の塩基配列の全長またはその一部を検出する P C R 用のプライマーのうち、少なくとも何れか一つを含むことを特徴とする造血器腫瘍細胞検出キット。

【請求項 1 6】

造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、

造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 遺伝子を挟み込む 2 つのマイクロサテライト・マーカの少なくとも一方の全長またはその一部を検出する P C R 用のプライマーを含むことを特徴とする造血器腫瘍細胞検出キット。 40

【請求項 1 7】

さらに、P C R 用試薬、および、制限酵素反応用試薬の少なくとも一方を含むことを特徴とする請求項 1 4、1 5 または 1 6 に記載の造血器腫瘍細胞検出キット。

【発明の詳細な説明】

【 0 0 0 1 】

【発明の属する技術分野】

本発明は、造血器腫瘍細胞検出方法および該検出方法に好適に用いられる造血器腫瘍細胞検出キットに関するものであり、特に、例えば、悪性リンパ腫や白血病等に特異的なプロテインチロシンホスファターゼ S H P 1 遺伝子産物の発現減少あるいは消失またはこれを 50

コードする S H P 1 遺伝子のメチル化を検出することによって、造血器腫瘍細胞を高感度かつ高特異的に検出できる検出方法および検出キットに関するものである。

【 0 0 0 2 】

【従来の技術】

ヒト ( H o m o s a p i e n s ) における悪性リンパ腫や白血病等の造血器腫瘍 ( 血液系の腫瘍 ) には、難治性で極めて予後の悪いものから比較的予後のよいものまで様々な種類が知られている。この造血器腫瘍の治療には、各種化学療法や放射線療法、あるいは免疫療法といった種々の療法がすでに実用化されているが、このような治療の結果、ほぼ腫瘍細胞が退縮したとしても、わずかに腫瘍細胞が生存していれば造血器腫瘍の再発は免れない。

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【 0 0 0 3 】

上記造血器腫瘍の診断は、従来では、複数の診断手法を併用することにより総合的に実施されている。具体的には、末梢血や各種生検材料を用いて、組織染色や免疫染色等による形態学的な観察や組織学的な観察が実施されたり、さらには、種々の分子生物学的解析や染色体解析等も実施されたりしている。また、上記造血器腫瘍の診断では、判定までにか

【 0 0 0 4 】

【発明が解決しようとする課題】

上記従来の各診断手法は、それ単独では造血器腫瘍細胞を高感度、高特異的、かつ迅速に検出できるものではない。それゆえ、これら従来の診断手法では、複数を併用して総合的に判断しなければ造血器腫瘍を診断することができない。

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【 0 0 0 5 】

つまり、従来の診断手法を使用する限り、複数の診断手法を併用しなければならないため、診断の煩雑化を招くだけでなく時間がかかり、造血器腫瘍細胞検出感度も特異性も高くないことから、医師の専門的な判断が診断に大きな比重を占めることになる。そのため、従来では、造血器腫瘍の診断技術は、実質的に医療現場での利用に限られており、各々の疾患には対応できるが、集団検診による造血器腫瘍の早期発見・早期治療を目的としては利用されていない。

【 0 0 0 6 】

造血器腫瘍細胞をより高感度かつ高特異的に実施するには、造血器腫瘍細胞に特異的であり、かつ広い範囲の造血器腫瘍に見られる感度の高いマーカーを用いることが考えられる。このようなマーカーを用いれば、造血器腫瘍の早期発見・診断を容易かつ迅速に実施することができ、医療上、悪性リンパ腫や白血病等の早期治療や再発予防に応用することが可能となるだけでなく、臨床検査産業や医薬品産業等にも利用可能な診断技術とすることができ、産業の発展に寄与することが可能となる。しかしながら、このようなマーカーは現在までのところ知られていない。

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【 0 0 0 7 】

本発明は上記課題に鑑みなされたものであって、その目的は、分子生物学的知見を利用して、迅速且つ簡便に造血器腫瘍細胞を高感度かつ高特異的に微量の患者検体から検出し造血器腫瘍の早期発見・診断および早期治療を容易にし、集団検診にも適用可能な造血器腫瘍細胞検出方法および検出キットを提供することにある。

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【 0 0 0 8 】

【課題を解決するための手段】

本発明者らは、上記課題に鑑み鋭意検討した結果、広い範囲の悪性の造血器腫瘍では、プロテインチロシンホスファターゼ S H P 1 蛋白質の発現抑制が極めて高頻度で見られ、しかも悪性度の高い造血器腫瘍において、上記 S H P 1 蛋白質の発現抑制の傾向が強くなることを見出し、S H P 1 遺伝子産物およびこれをコードする S H P 1 遺伝子の双方をマーカーとして用いることで、造血器腫瘍細胞の高感度、高特異的、かつ短時間に検出でき、かつ産業上利用できる造血器腫瘍細胞検出技術を実現し得ることを見出し、本発明を完成するに至った。

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## 【 0 0 0 9 】

すなわち、本発明にかかる造血器腫瘍細胞検出方法は、上記の課題を解決するために、( 1 ) 造血器細胞を含む検体試料中に含まれる、造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質および S H P 1 m R N A の少なくとも一方の発現量を定量する S H P 1 遺伝子産物定量工程、( 2 ) 上記検体試料から得られる、S H P 1 遺伝子の塩基配列中に含まれる C p G 島のメチル化を確認する S H P 1 遺伝子メチル化確認工程、および( 3 ) 上記検体試料に含まれる S H P 1 遺伝子の異型接合性喪失( L O H ) の有無を確認する S H P 1 遺伝子 L O H 確認工程、の少なくとも一方を含むことを特徴としている。

## 【 0 0 1 0 】

上記 S H P 1 遺伝子産物の発現抑制は、悪性の造血器腫瘍細胞に極めて高頻度で見られるのに対し、正常な血液細胞にはこの現象が見られない。また、上記 S H P 1 蛋白質の発現抑制は、S H P 1 遺伝子のメチル化によるものである。さらに、DNAメチル化による S H P 1 遺伝子の転写抑制の前後には、S H P 1 遺伝子の一つの対立遺伝子が喪失している。

## 【 0 0 1 1 】

上記方法によれば、上記知見を利用して、検体試料から得られる S H P 1 遺伝子のメチル化を確認し、造血器腫瘍細胞の存在を検出することで、悪性の造血器腫瘍細胞の存在の有無をスクリーニングし、一方検体試料中の S H P 1 遺伝子産物、具体的には S H P 1 蛋白質、または S H P 1 m R N A、あるいはその両方の発現を定量する。

## 【 0 0 1 2 】

すなわち、上記方法では、S H P 1 遺伝子の不活性化を、遺伝子 D N A の修飾と m R N A と蛋白質と対立遺伝子の喪失という最大で四重のマーカを用いて判定できることになる。すなわち、S H P 1 遺伝子の発現低下という一つの造血器腫瘍細胞特異的な現象を4段階で確認することができるため、非常に高い特異性で造血器腫瘍細胞を検出することができる。

## 【 0 0 1 3 】

本発明にかかる造血器腫瘍細胞検出方法の好ましい一例としては、上記 S H P 1 遺伝子メチル化確認工程に、上記検体試料から得られた遺伝子試料を、シトシンを含む塩基配列を認識するメチル化感受性制限酵素で処理する遺伝子切断試行段階と、上記メチル化感受性制限酵素で処理された遺伝子に対して、上記 S H P 1 遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識切断される塩基配列を含む領域を増幅するプライマーを用いて P C R 法を実施する遺伝子増幅試行段階と、増幅された特定のサイズの遺伝子の量を確認する遺伝子増幅量確認段階とが含まれる検出方法を挙げることができる。

## 【 0 0 1 4 】

上記方法によれば、メチル化感受性制限酵素を用いて検体試料から得られた遺伝子試料に含まれる S H P 1 遺伝子の切断を試みることでメチル化の有無を区別し、さらに P C R を用いて増幅してから、得られる特定サイズの P C R 産物の量を確認する。それゆえ、検体試料から微量の S H P 1 遺伝子さえ得られれば、S H P 1 遺伝子のメチル化を検出することができる。そのため、検体試料中に造血器腫瘍細胞がごく微量しか存在していなくても高い検出感度で、しかも高特異的に造血器腫瘍細胞を検出することが可能となる。

## 【 0 0 1 5 】

上記検出方法においては、上記プライマーが、さらに、配列番号 1 または 2 に示す塩基配列に含まれる部分塩基配列、またはこの部分塩基配列と相補性を有するポリヌクレオチドであることが好ましい。

## 【 0 0 1 6 】

また、上記検出方法においては、上記遺伝子増幅量確認段階では、電気泳動法を用いて特定サイズの遺伝子の量を確認することが好ましい。

## 【 0 0 1 7 】

さらに、上記検出方法においては、上記遺伝子切断試行段階では、メチル化感受性制限酵

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素として、同一の塩基配列を認識するメチル化非感受性制限酵素が知られている制限酵素を用いることが好ましい。

【 0 0 1 8 】

本発明にかかる造血器腫瘍細胞検出方法の好ましい他の一例としては、上記 S H P 1 遺伝子メチル化確認工程に、上記検体試料から得られる遺伝子試料を、重亜硫酸塩で処理する遺伝子修飾段階と、重亜硫酸塩で処理された遺伝子試料に含まれる、S H P 1 遺伝子の塩基配列中のメチル化シトシンの有無を判定するメチル化シトシン含有判定段階とが含まれる検出方法を挙げることができる。

【 0 0 1 9 】

上記方法によれば、重亜硫酸塩を用いて検体試料から得られた遺伝子試料を処理すると、塩基配列中のシトシンはウラシルに変換されるが、メチル化されたシトシンは変換されない。そのため、遺伝子修飾段階後の S H P 1 遺伝子の塩基配列中にシトシンが含まれるか否かを判定するのみで、S H P 1 遺伝子のメチル化を検出することができる。そのため、簡素なメカニズムで高特異的に造血器腫瘍細胞を検出することが可能となる。

【 0 0 2 0 】

上記検出方法においては、上記メチル化シトシン含有判定段階では、P C R によりメチル化シトシンを検出する方法、遺伝子の塩基配列の決定によりメチル化シトシンを検出する方法、またはメチル化シトシンを含む塩基配列を識別する方法による遺伝子の処理のうち、少なくとも何れかが用いられても好ましい。

【 0 0 2 1 】

上記方法によれば、少なくとも P C R を用いることで、検体試料から微量の S H P 1 遺伝子さえ得られれば、S H P 1 遺伝子のメチル化を検出することができる。そのため、検体試料中に造血器腫瘍細胞がごく微量しか存在していなくても高い検出感度で高特異的に造血器腫瘍細胞を検出することが可能となる。

【 0 0 2 2 】

上記検出方法においては、上記遺伝子修飾段階では、重亜硫酸塩として、重亜硫酸ナトリウムが用いられることが好ましい。また、上記遺伝子修飾段階では、重亜硫酸塩とともに尿素が併用されてもよい。

【 0 0 2 3 】

本発明にかかる造血器腫瘍細胞検出方法においては、上記何れの例の検出方法であっても、上記 S H P 1 遺伝子産物定量工程では、S H P 1 蛋白質を抗原とする S H P 1 抗体を用いて S H P 1 蛋白質を定量すると好ましい。具体的には、上記 S H P 1 遺伝子産物定量工程では、酵素抗体法（免疫組織化学法、免疫細胞化学法、E L I S A ( e n z y m e - l i n k e d i m m u n o s o r b e n t a s s a y ) 法）またはウエスタンブロッティング法により S H P 1 蛋白質が定量されると好ましい。

【 0 0 2 4 】

上記方法によれば、抗原抗体反応を利用して S H P 1 蛋白質を定量することになるので、簡素なメカニズムで高特異的に造血器腫瘍細胞を検出することが可能となる。

【 0 0 2 5 】

あるいは、本発明にかかる造血器腫瘍細胞検出方法においては、上記何れの例の検出方法であっても、上記 S H P 1 遺伝子産物定量工程では、配列番号 3 に示す S H P 1 遺伝子 c D N A の塩基配列の全長またはその一部を検出するポリヌクレオチドを用いて S H P 1 遺伝子の m R N A の発現を検出することにより、S H P 1 m R N A を定量しても好ましい。具体的には、上記 S H P 1 遺伝子産物定量工程では、ノーザンブロッティング法、逆転写 P C R 法、リアルタイム逆転写 P C R 法、または R N A i n s i t u ハイブリダイゼーション法により S H P 1 遺伝子の m R N A の発現が検出されると好ましい。

【 0 0 2 6 】

上記方法によれば、S H P 1 遺伝子産物として S H P 1 遺伝子の m R N A により S H P 1 遺伝子産物を定量することになるので、S H P 1 遺伝子の c D N A と相同性を有するオリゴペプチドをプローブやプライマーとして利用することで、簡素なメカニズムで高特異

的かつ高感度に造血器腫瘍細胞を検出することが可能となる。

【0027】

本発明にかかる造血器腫瘍細胞検出方法の好ましいさらに他の一例としては、異型接合性喪失の有無の確認は、上記SHP1遺伝子を挟み込む2つのマイクロサテライト・マーカの少なくとも一方、または、上記SHP1遺伝子中か、その近辺に存在する単一塩基多型のような遺伝子多型を、PCRを用いたフラグメント解析することにより実施される方法を挙げることができる。このとき用いられる検体試料は、造血器細胞を含む検体試料であればよい。また、対照としては、血液学的完全寛解後に得られる検体を用いてもよい、他の正常組織細胞を用いてもよい。

【0028】

上記の方法によれば、マイクロサテライト・マーカまたは単一塩基多型(SNP)等の遺伝子多型の異型接合性喪失をPCRにより確認することによって、SHP1遺伝子の異型接合性喪失を確認しているのので、簡素なメカニズムでより確実に造血器腫瘍細胞を検出することが可能となる。

【0029】

本発明にかかる造血器腫瘍細胞検出キットの好ましい一例としては、造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、(1)造血器細胞に特異的なプロテインチロシンホスファターゼSHP1蛋白質を抗原とするSHP1抗体、および、(2)シトシンを含む塩基配列を認識するメチル化感受性制限酵素と、SHP1遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識切断される塩基配列を含む領域を増幅するPCR用のプライマーと、上記SHP1遺伝子のメチル化陽性及びメチル化陰性対照DNAとのうち、少なくとも一方を含む構成を挙げることができる。

【0030】

あるいは、本発明にかかる造血器腫瘍細胞検出キットの好ましい他の一例としては、造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、(1)上記SHP1抗体、および(2)遺伝子処理レベルまで精製された重亜硫酸塩と、該重亜硫酸塩で処理された遺伝子試料に含まれるSHP1遺伝子の塩基配列中のシトシンの有無の判定用プライマー、および、(3)配列番号3に示すSHP1遺伝子cDNAの塩基配列の全長またはその一部と相同性を持つPCR用のプライマーのうち、少なくとも何れか一つを含む構成を挙げることができる。

【0031】

さらには、本発明にかかる造血器腫瘍細胞検出キットの好ましいさらに他の一例としては、造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、造血器細胞に特異的なプロテインチロシンホスファターゼSHP1遺伝子を挟み込む2つのマイクロサテライト・マーカの少なくとも一方の全長またはその一部を検出するPCR用のプライマーを含む構成を挙げることができる。

【0032】

上記造血器腫瘍細胞検出キットにおいては、さらに、PCR用試薬、および、制限酵素反応用試薬の少なくとも一方を含むことが好ましい。

【0033】

上記何れの構成であっても、前述した造血器腫瘍細胞検出方法を実施するために好ましい薬剤や標本等が含まれている。そのため、上記検出キットを用いることで、本発明にかかる造血器腫瘍細胞検出方法を容易かつ迅速に実施することができ、本発明を臨床検査産業や医薬品産業等の産業レベルで利用することが可能となる。

【0034】

【発明の実施の形態】

【実施の形態1】

本発明における実施の一形態について図1ないし図24に基づいて説明すれば以下の通りである。なお、本発明はこれに限定されるものではない。

【0035】

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本発明は、造血器細胞を含む検体試料中に含まれる、造血器細胞に特異的なプロモーターからのプロテインチロシンホスファターゼSHP1遺伝子産物すなわちSHP1蛋白質とmRNAとを定量するとともに、上記検体試料から得られる、SHP1遺伝子の塩基配列中に含まれるCpG島のメチル化を確認することで、上記検体試料中から造血器腫瘍細胞を検出する技術である。

#### 【0036】

本発明で、造血器腫瘍細胞を検出するためのマーカーとして用いられるSHP1遺伝子は、染色体12p13に存在し、図1～図10および配列番号1に示す塩基配列をゲノムDNA（ワイルドタイプ）のセンス鎖とし、図11～図20および配列番号2に示す塩基配列をアンチセンス鎖とする16のエキソン（図および配列表中大文字で示す領域）を有する遺伝子である。そのcDNAは、図21および配列番号3に示す塩基配列を有する約1.8kbのサイズを有している。なお、SHP1遺伝子はSH-PTP1、PTP1C、HCP、HCPH、PTPN6、HPTP1C、SHP-1Lと同一の遺伝子である。

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#### 【0037】

上記SHP1遺伝子にコードされているSHP1蛋白質は、分子量68kDで、各種造血器細胞に特異的なプロテインチロシンホスファターゼ（PTPase）であり、図22に示すように、N末端側にタンデム構造となる2つのSH2（Src homology domain 2）領域（270アミノ酸残基）と、246アミノ酸残基のPTPaseドメインと、93アミノ酸残基のC末端側領域とを有する構造となっている。また、図23および配列番号4に示すアミノ酸配列を有している。

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#### 【0038】

ヒトの造血器腫瘍、例えば悪性リンパ腫や白血病では、多くの種類で90%以上の高い頻度でSHP1蛋白質の強い発現抑制が見られる（例えば、American Journal of Pathology, Vol. 159, No. 4, October 2001: 1495-1505等参照）。このように悪性の造血器腫瘍細胞では、上記SHP1蛋白質の発現抑制が極めて高頻度で見られるのに対し、正常な血液細胞にはこの現象が見られない。

#### 【0039】

本発明者らは、上記SHP1蛋白質の発現抑制が、上記SHP1遺伝子がメチル化されることによる転写異常を原因とすることを独自に見出した。

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#### 【0040】

例えば、図24に示すように、図1～図10および配列番号1に示すゲノムDNA（ワイルドタイプ）のセンス鎖（181塩基～2160塩基まで例示）において、1001塩基～1163塩基のエキソン（図中大文字）の前にプロモーター領域が存在するが、この近傍にシトシン（C）とグアニン（G）とが並ぶCG配列が多く存在しCpG島（CpG island）を形成している（図24中ではCG配列を太字の網掛けで示す）。正常な造血器細胞では、このCpG島のシトシンはメチル化されていないが、例えば悪性のリンパ腫細胞では、上記CG配列のシトシンの多くがメチル化されている。勿論、このCG配列におけるシトシンのメチル化はセンス鎖のみならずアンチセンス鎖にも同じように生じる。

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#### 【0041】

上記CpG島におけるCG配列の高度なメチル化は、SHP1遺伝子のDNAからmRNAの転写を阻害し、その結果、SHP1蛋白質の生産が抑制される。この現象は、上述したように造血器腫瘍細胞では極めて高頻度に見られる。しかも、各種造血器腫瘍患者の完全寛解期には、SHP1遺伝子におけるDNAのメチル化が完全に消失し、分子生物学上の知見と臨床上の知見との間に非常に高い相関関係が見られる。それゆえ、メチル化によるSHP1遺伝子の発現抑制が、造血器腫瘍細胞の発症機構の中で重要な役割を果たしていることが推測される。そこで本発明では、上記SHP1遺伝子の発現抑制という現象を、造血器腫瘍細胞のマーカーとして利用する。

#### 【0042】

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さらに、本発明者らは、悪性リンパ腫や白血病等の疾患が発症する際に、上述した、DNAメチル化によってSHP1遺伝子の転写抑制が生じる前後に、SHP1遺伝子の一つの対立遺伝子が喪失することも独自に見出した。そこで、SHP1遺伝子の異型接合性喪失を確認することによって、SHP1遺伝子の対立遺伝子の喪失を確認することが可能となる。それゆえ、SHP1遺伝子の異型接合性喪失も造血器腫瘍細胞のマーカーとして利用することができる。

【 0 0 4 3 】

悪性リンパ腫や白血病において、SHP1遺伝子には、高頻度のDNAメチル化、高頻度の異型接合性喪失、SHP1遺伝子の発現の低下または消失が検出され、さらには、外来SHP1遺伝子導入が血球系の細胞の増殖を抑制する傾向にある。これにより、SHP1 10  
遺伝子が癌抑制遺伝子の一つであることが強く示唆される。

【 0 0 4 4 】

そこで、本発明では、SHP1遺伝子メチル化確認工程で、上記検体試料から得られるSHP1遺伝子の塩基配列中に含まれるCpG島のメチル化を確認し、SHP1遺伝子産物定量工程にて、造血器細胞を含む検体試料中に含まれるSHP1蛋白質およびmRNAの少なくとも一方を定量し、さらに、SHP1遺伝子LOH確認工程で、SHP1遺伝子の異型接合性喪失を確認するという三つの工程を利用する。これら工程は単独で用いられても良いし、双方ともに用いられても良い。さらに、SHP1遺伝子産物定量工程では、SHP1蛋白質のみ定量されても良いし、SHP1mRNAのみ検出されても良いし、双方ともに検出されてもよい。 20

【 0 0 4 5 】

これによって、例えば、まず、検体試料中のSHP1遺伝子のメチル化を検出することでスクリーニングし、その後、検体試料のSHP1遺伝子産物の発現をSHP1mRNAおよびSHP1蛋白質の少なくとも一方で定量することで、悪性の造血器腫瘍細胞の有無を確認することで造血器腫瘍細胞の存在を確定するという検出プロセスを実施することができる。

【 0 0 4 6 】

したがって、本発明では、SHP1遺伝子の発現を、遺伝子DNAの修飾とmRNAと蛋白質と対立遺伝子の喪失という最大で四重のマーカーを用いて判定できることになる。すなわち、SHP1遺伝子の発現低下という一つの造血器腫瘍細胞特異的な現象を3段階で 30  
確認することができるため、非常に高い特異性で造血器腫瘍細胞を検出することができる。

【 0 0 4 7 】

また、上述したように、本発明におけるSHP1遺伝子を導入することで、血球系の細胞の増殖を抑制する傾向にあることも確認されている。それゆえ、SHP1遺伝子は、遺伝子治療に用いることも可能であり、例えば、腫瘍細胞にSHP1遺伝子の発現ベクターをトランスフェクトすることにより、腫瘍細胞の増殖を抑制することが期待できる。

【 0 0 4 8 】

本発明で用いられる検体試料は、末梢血あるいは骨髓液等の造血器細胞を含む検体試料であればどのような検体試料であっても特に限定されるものではない。本発明における造血器細胞とは、各種血液細胞を含むが、特に好ましくは各種白血球が挙げられる。より具体的には、リンパ球（T細胞・B細胞）、顆粒球（好中球、好酸球、好塩基球）、単球並びにマクロファージ、マスト細胞、ナチュラルキラー細胞等を挙げることができる。あるいは造血幹細胞やリンパ球幹細胞であってもよい。 40

【 0 0 4 9 】

したがって、本発明で用いられる検体試料には、上記造血器細胞が含まれている血液や骨髓液あるいは体液等をヒトから採取し、これをそのまま検体試料として用いてもよいし、採取した血液や体液に対して従来公知の処理を施すことによって、分子生物学的な分析を実施し易い分析用検体試料としてもよい。

【 0 0 5 0 】

本発明が適用可能な造血器腫瘍としては、具体的には、例えば、慢性骨髄性白血病、フィラデルフィア染色体ポジティブ（+（9；22）（q q 3 4；q 1 1），BCR／ABL）慢性骨髄性白血病、慢性好中球白血病、慢性好酸球白血病／高好酸球症候群、慢性突発性骨髄繊維症、真性多血症、本態性血小板増加症、その他分類できない骨髄増殖性疾患等の各種骨髄増殖性疾患；

慢性骨髄性単球白血病、非定型慢性骨髄性白血病、幼年性骨髄性単球白血病等の骨髄異型性／骨髄増殖性疾患；

環状鉄芽球を伴う難治性貧血、環状鉄芽球を伴わない難治性貧血、多系列異形成を伴う難治性血球減少症（骨髄異型性症候群）、過剰芽球5q-症候群を伴う難治性貧血（骨髄異型性症候群）、その他分類できない骨髄異型性症候群等の骨髄異型性症候群；

再発性細胞遺伝学的転座を伴う急性骨髄性白血病（AML）（例えば、+（8；21）（q 2 2；q 2 2）を伴うAML、AML 1（CBF- $\alpha$ ）／ETO、急性前骨髄性白血病（+（15；17）（q 2 2；q 1 1-1 2）を伴うAMLおよびその変形、PML／RAR- $\alpha$ ）、異常な骨髄好酸球（inv（16）（p 1 3 q 2 2）あるいは+（16；16）（p 1 3；q 1 1）、CBF $\beta$ ／MYH11X）を伴うAML、11q 2 3（MLL）異常を伴うAML、前骨髄異型性症候群を伴いかつ多系列異形成を伴うAML、前骨髄異型性症候群を伴いかつ多系列異形成を伴わないAML、治療に関係するAMLおよび骨髄異型性症候群（アルキル化剤に関係する治療、エピドフィロトキシンに関係する治療、あるいはその他のタイプの治療）、他に部門に属さないAML（低分化型、成熟を伴わないもの、成熟を伴うもの、急性骨髄性単球白血病、急性単球白血病、急性赤芽球白血病、急性巨核球白血病、急性好塩基球白血病、骨髄繊維症を伴う急性汎骨髄過剰増殖症）、急性二形質性白血病等の急性骨髄性白血病（AML）；

前駆体B細胞性腫瘍（前駆体B-リンパ芽球性白血病／リンパ腫（前駆体B細胞急性リンパ芽球性白血病）、成熟（末梢）B細胞性腫瘍（B細胞慢性リンパ球性白血病／小リンパ球性リンパ腫、B細胞前リンパ球性白血病、リンパ形質細胞性リンパ腫、脾辺縁領域B細胞リンパ腫（+／-絨毛リンパ球）、毛状細胞白血病、形質細胞性骨髄腫（形質細胞腫）、MAL T型節外辺縁型B細胞リンパ腫、節性辺縁型B細胞リンパ腫（+／-単球型B細胞）、濾胞性リンパ腫、マントル細胞リンパ腫、びまん性大型B細胞リンパ腫（縦隔大細胞B細胞リンパ腫、原発性滲出リンパ腫）、Burkittリンパ腫／Burkitt細胞白血病）等のB細胞性腫瘍；

前駆体T細胞性腫瘍（前駆体T-リンパ芽球性白血病／リンパ腫（前駆体T細胞急性リンパ芽球性白血病）、成熟（末梢）T細胞性腫瘍（T細胞前リンパ球性白血病、T細胞顆粒リンパ球白血病、侵襲型NK細胞白血病、成人T細胞リンパ腫・白血病（HTLV1+）、鼻型節外性NK／T細胞リンパ腫、腸管症型T細胞リンパ腫、肝脾型 $\gamma$ - $\delta$ T細胞リンパ腫、皮下蜂窩織炎様T細胞リンパ腫、菌状息肉腫／Sezary症候群、退形成性大型細胞リンパ腫（T／ヌル細胞、原発性皮膚未分化型）、他に部門に属さない末梢T細胞リンパ腫、血管免疫芽球T細胞リンパ腫）等のT細胞およびNK細胞性腫瘍；

節性リンパ球優勢ホジキンリンパ腫、古典的ホジキンリンパ腫（結節硬化ホジキンリンパ腫（等級1および2）、リンパ球リッチ古典的ホジキンリンパ腫、混合細胞型ホジキンリンパ腫、リンパ球枯渇ホジキンリンパ腫）等のホジキンリンパ腫（ホジキン病）；

等を挙げることができるが、特に限定されるものではない。

#### 【0051】

本発明におけるSHP1遺伝子産物定量工程は、検体試料中のSHP1蛋白質およびSHP1 mRNAの少なくとも一方を定量できる方法であれば特に限定されるものではないが、具体的には、SHP1蛋白質を抗原とするSHP1抗体を用いてSHP1蛋白質を定量する方法（蛋白質定量法）か、SHP1遺伝子のmRNAの発現を検出することにより、SHP1 mRNAを定量する方法（mRNA定量法）を好適に用いることができる。

#### 【0052】

まず、上記蛋白質定量法のより具体的な手法としては、SHP1抗体を用いたウエスタンブロッティング法または酵素抗体法（Immunochemistry）（免疫組織化学

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法、免疫細胞化学法、E L I S A ( e n z y m e - l i n k e d i m m u n o s o r b e n t a s s a y ) 法) を挙げることができる。

【 0 0 5 3 】

上記蛋白質定量法で用いられるSHP1抗体は、図22、図23および配列番号4に示す構造を有するSHP1蛋白質の少なくとも一部の構造を抗原決定基として認識し、SHP1蛋白質を免疫学的に確実に検出できる抗体であれば特に限定されるものではなく、ポリクローナル抗体であってもよいし、モノクローナル抗体であってもよい。

【 0 0 5 4 】

上記SHP1抗体は、従来公知の方法で製造してもよいし、市販のSHP1抗体を用いてもよい。SHP1抗体の製造方法としては、例えば、モノクローナル抗体であれば、SHP1蛋白質で免疫したマウス脾臓リンパ球とマウスの骨髓細胞とを融合させてなるハイブリドーマにより産生する手法が挙げられる。また、上記SHP1抗体がポリクローナル抗体であれば、SHP1蛋白質で免疫したウサギの免疫血清から精製する手法が挙げられる。また、市販のSHP1抗体としては、#SH-PTP1(D-11):sc7289および#SH-PTP1(C-19):sc287(Santa Cruz Biotechnology Inc. 製)、#anti SHPTP(06117)および#anti mouse SHPTP(05281) )

(Upstate Biotechnology Inc. 製) 等が挙げられる。

【 0 0 5 5 】

上記SHP1抗体を用いた酵素抗体法(免疫組織化学法、免疫細胞化学法、ELISA法)は、従来公知の方法(例えば、『酵素抗体法』渡辺慶一・中根一穂編集、学際企画出版(昭和61年)や、Brown R. W. et al: Modern Pathol. 199; 8(5):515-20(1995)等の文献に開示されている方法)を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。

【 0 0 5 6 】

同様に、上記SHP1抗体を用いたウエスタンブロッティング法も、従来公知の方法(例えば、『実験操作ブロッティング法』日野嘉幸他編、ソフトサイエンス社(昭和62年)や、Towbin H. et al: Proc. Natl. Acad. Sci. USA 76, 4350, (1979) 等の文献に開示されている方法)を好適に用いること

【 0 0 5 7 】

上記蛋白質定量法を用いることで、抗原抗体反応を利用してSHP1蛋白質を定量することになる。そのため、簡素なメカニズムで高特異的に造血器腫瘍細胞を検出することが可能となる。

【 0 0 5 8 】

次に、上記mRNA定量法のより具体的な手法としては、配列番号3(図21参照)に示すSHP1遺伝子cDNAの塩基配列の全長またはその一部と相同性を有するポリヌクレオチドを用いてSHP1遺伝子のmRNAの発現を検出する方法が挙げられ、より具体的には、ノーザンブロッティング法、逆転写ポリメラーゼ連鎖反応法(RT-PCR)、リアルタイム逆転写ポリメラーゼ連鎖反応法(real time RT-PCR)、またはRNA in situハイブリダイゼーションを挙げることができる。

【 0 0 5 9 】

上記ノーザンブロッティング法、RT-PCR、real time RT-PCR、およびRNA in situハイブリダイゼーションの何れの方法も従来公知の方法(例えば、"Molecular cloning" a laboratory manual, Sambrook J., Russell DW., Cold Spring Harbor Lab Press. (2001)や、"Current protocols in molecular biology" edited by Ausubel FM et al., John Wiley & Sons Inc. (2

001) 等の文献に開示されている方法)を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。

【0060】

上記ノーザンブロッティング法やRNA in situ ハイブリダイゼーションでは、原理的には、配列番号3に示すSHP1遺伝子のcDNAの全長あるいはその一部をプローブとして用いることができる。また、RT-PCRやreal time RT-PCRでも、原理的には、配列番号3に示すSHP1遺伝子のcDNAの一部と相同性を有するオリゴヌクレオチドをプライマーとして用いることができる。具体的には、例えば、後述する実施例3や実施例4に示すプライマーペアを用いることができる。

【0061】

それゆえ、mRNA定量法では、配列番号3に示すSHP1遺伝子cDNAの塩基配列の全長またはその一部と相同性を有するポリヌクレオチドを用いてSHP1遺伝子のmRNAの発現を検出すればよい。

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【0062】

上記mRNA定量法を用いることで、SHP1遺伝子の転写産物であるSHP1mRNAを定量することになるので、SHP1遺伝子のcDNAと相同性を有するポリヌクレオチドをプローブやプライマーとして利用することで、簡素なメカニズムで迅速、高特異的かつ高感度に造血器腫瘍細胞を検出することが可能となる。

【0063】

本発明におけるSHP1遺伝子メチル化確認工程は、検体試料から得られるSHP1遺伝子の塩基配列中に含まれるCpG島のメチル化を確認できる方法であれば特に限定されるものではないが、本実施の形態では、例えば、遺伝子切断試行段階と、遺伝子増幅試行段階と、遺伝子増幅量確認段階とを含むメチル化感受性制限酵素を利用した方法(以下、説明の便宜上、制限酵素確認法と称する)を好適に用いることができる。

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【0064】

本実施の形態で用いられるメチル化感受性制限酵素とは、二本鎖DNAにおいて認識対象となる塩基配列にシトシンを含んでおり、かつ、この塩基配列中のシトシンがメチル化された場合には、該塩基配列の二本鎖DNAを切断できない制限酵素であれば特に限定されるものではない。

【0065】

上記メチル化感受性制限酵素としては、具体的には、例えば、HpaII、EagIまたはNaeI等を挙げることができる。中でも、HpaIIをより好ましく用いることができる。HpaIIは、CCGGの塩基配列を認識して二本鎖DNA切断するエンドヌクレアーゼであるが、同じ塩基配列を認識して二本鎖DNAを切断する制限酵素として、MspIが知られている。

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【0066】

上述したように、HpaIIはメチル化されたCCGGの塩基配列の二本鎖DNAを切断できないが、MspIはメチル化の有無に関わらずCCGGの塩基配列を認識して二本鎖DNAを切断することが可能である。すなわち、MspIはメチル化非感受性制限酵素である。それゆえ、HspIIとMspIとを併用することで、後述するように、検体試料中のSHP1遺伝子の切断を確実に確認するためのコントロールとして利用することが可能になり、本実施の形態における制限酵素確認法の信頼性をより一層向上させることができる。

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【0067】

このように、本実施の形態における制限酵素確認法では、使用するメチル化感受性制限酵素と同じ塩基配列を認識するメチル化非感受性制限酵素をコントロールとして使用することが好ましい。勿論、メチル化感受性およびメチル化非感受性制限酵素の組み合わせは上記HspII・MspIに限定されるものではないことは言うまでもない。

【0068】

次に、本実施の形態におけるSHP1遺伝子メチル化確認工程、すなわち制限酵素確認法 50

による S H P 1 遺伝子のメチル化の確認について具体的に説明する。

【 0 0 6 9 】

まず、遺伝子切断試行段階として、造血器細胞を含む前記検体試料から得られた遺伝子試料を、シトシンを含む塩基配列を認識する上記メチル化感受性制限酵素で処理する。この段階では、メチル化感受性制限酵素の処理により遺伝子試料中に含まれる S H P 1 遺伝子の切断を試みる。すなわち、前記検体試料中に含まれる造血器細胞が正常な細胞のみであれば、S H P 1 遺伝子は切断されるが、造血器腫瘍細胞が含まれていれば、S H P 1 遺伝子は C G 配列がメチル化されているため切断されない。

【 0 0 7 0 】

前記検体試料から遺伝子試料を調製する方法は従来公知の方法を用いることができ特に限定されるものではない。また、調製された遺伝子試料は、S H P 1 遺伝子を含んでいればよく、制限酵素処理や P C R 等を阻害しない限り他の成分が含まれていても良い。それゆえ、前記検体試料中に含まれる造血器細胞やその他の細胞から抽出される各種 D N A や R N A の混合物であればよい。また、メチル化感受性制限酵素による処理についても特に限定されるものではなく、該メチル化感受性制限酵素の種類や調製された遺伝子試料の状態等に応じて、適宜条件等を設定すればよい。

【 0 0 7 1 】

次に、遺伝子増幅試行段階として、上記メチル化感受性制限酵素で処理された遺伝子試料に対して、上記 S H P 1 遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識切断される塩基配列を含む領域を増幅するプライマーを用いて、P C R を実施する。この段階では、メチル化感受性制限酵素で処理した制限酵素処理物を、上記プライマーを用いて P C R 処理することにより、S H P 1 遺伝子のみの増幅を試みる。正常な S H P 1 遺伝子のみであれば、プライマーペアに挟まれる領域が切断されているため S H P 1 遺伝子は増幅できないが、メチル化されている S H P 1 遺伝子が含まれていれば、上記プライマーペアに挟まれる領域は切断されていないため S H P 1 遺伝子が増幅される。

【 0 0 7 2 】

上記遺伝子増幅試行段階で用いられる上記プライマーとしては、メチル化感受性制限酵素に認識される塩基配列を含む領域を増幅するポリヌクレオチドであればよい。それゆえプライマーの設計条件等についても特に限定されるものではない。基本的には、本実施の形態で用いられるプライマーペアは、メチル化感受性制限酵素に認識される上記塩基配列を含む領域の少なくとも外側に位置し、配列番号 1 または 2 ( 図 1 ~ 図 1 0 および図 1 1 ~ 図 2 0 参照 ) に示す S H P 1 遺伝子の塩基配列に含まれる部分塩基配列、またはこの部分塩基配列と相補性を有するポリヌクレオチドであればよく、その場所やサイズ等については特に限定されるものではない。

【 0 0 7 3 】

次に、遺伝子増幅量確認段階として、増幅された遺伝子の量を確認する。この段階では、S H P 1 遺伝子が増幅されたか否かを確認する。S H P 1 遺伝子が増幅されれば、元の検体試料中に造血器腫瘍細胞が含まれていることになる。

【 0 0 7 4 】

上記遺伝子増幅量確認段階で用いられる S H P 1 遺伝子の有無の確認方法としては特に限定されるものではないが、電気泳動法を用いてマーカと比較することにより遺伝子の増幅量を確認する手法が最も一般的で確立された手法であるため好ましく用いることができる。また、電気泳動後に得られた D N A バンドをメンブレンにブロッティングして検出してもよい。

【 0 0 7 5 】

上記遺伝子増幅量確認段階で用いられる S H P 1 遺伝子の有無の確認方法としては特に限定されるものではないが、検体試料と同時にメチル化陽性およびメチル化陰性対照 D N A を用いて反応を行った後電気泳動法を用いて遺伝子の増幅量を確認する手法が最も一般的で確立された手法であるため好ましく用いることができる。また、電気泳動後に得られた D N A バンドをメンブレンにブロッティングして検出してもよい。

## 【 0 0 7 6 】

上記 S H P 1 遺伝子のメチル化陽性およびメチル化陰性対照 D N A は、S H P 1 遺伝子を用いたものであればよく、特に限定されるものではない。具体的には、メチル化感受性制限酵素またはメチル化非感受性制限酵素により処理することで得られる、増幅量を比較できる程度の濃度を有する D N A 溶液を挙げることができる。

## 【 0 0 7 7 】

さらに、制限酵素確認法による S H P 1 遺伝子メチル化確認工程では、コントロールとして、メチル化感受性制限酵素による処理と並行して、同一の検体試料をメチル化非感受性制限酵素で処理して、それを遺伝子増幅量確認段階で確認すると好ましい。すなわち、上記遺伝子切断試行段階では、メチル化感受性制限酵素として、同一の塩基配列を認識するメチル化非感受性制限酵素が知られている制限酵素を用いることが非常に好ましい。これによって、制限酵素確認法による S H P 1 遺伝子のメチル化の確実性を高めることができる。

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## 【 0 0 7 8 】

本発明における S H P 1 サテライト L O H 確認工程は、造血器細胞を含む検体試料において、この検体試料に含まれる S H P 1 遺伝子の異型接合性喪失 ( L o s s o f h e t e r o z y g o s i t y , L O H と略す) の有無を確認することができる方法であれば特に限定されるものではないが、具体的には、S H P 1 遺伝子を挟み込むマイクロサテライト・マーカー、または、上記 S H P 1 遺伝子中か、その近辺に存在する単一塩基多型 ( s i n g l e n u c l e o t i d e p o l y m o r p h i s m , S N P ) のような遺伝子多型 ( p o l y m o r p h i s m ) について、P C R を用いたフラグメント解析によって L O H を確認する方法を好適に用いることができる。

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## 【 0 0 7 9 】

上記 S H P 1 遺伝子の両側に存在するマイクロサテライト・マーカーや、S H P 1 遺伝子中またはその近辺に存在する遺伝子多型については、特に限定されるものではなく、どのようなマーカーを用いてもよいが、具体的には、例えば、D 1 2 S 3 3 6 マーカーおよび D 1 2 S 3 5 6 マーカーを挙げることができる。これらマーカーの塩基配列は、インターネット・ゲノム・データベース ( U R L : h t t p : / / g d b w w w . g d b . o r g . / ) から得られる。これらマーカーのうち、D 1 2 S 3 5 6 マーカーはテロメア側に存在し、S H P 1 遺伝子から約 4 . 4 c M の距離にある。一方、D 1 2 S 3 3 6 マーカーはセントロメア側に存在し、S H P 1 遺伝子から約 2 . 4 c M の距離にある。

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## 【 0 0 8 0 】

検体試料における S H P 1 遺伝子の L O H (異型接合性喪失) の確認に際しては、S H P 1 サテライト L O H 確認工程で用いられる検体試料は、造血器細胞を含む検体試料となっていればよい。また、L O H の具体的な方法は特に限定されるものではないが、後述する実施例 6 に示すように、P C R 反応によって上記各マーカーの少なくとも一方の全長またはその一部を検出するマイクロサテライト解析を行えばよい。このときの P C R 反応他の条件も特に限定されるものではなく、P C R 用のプライマーとしては、例えば、D 1 2 S 3 3 6 マーカーまたは D 1 2 S 3 5 6 マーカーの少なくとも一部を検出できるようなプライマーであればよく、その他の条件についても適切な条件を適宜設定すればよい。

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## 【 0 0 8 1 】

本発明における S H P 1 サテライト L O H 確認工程で用いられる検体試料は、造血器細胞を含む検体試料であれば特に限定されるものではない。また、対照として用いる検体試料も特に限定されるものではなく、血液学的完全寛解後に得られる検体を用いてもよいし、他の正常組織細胞を用いてもよい。

## 【 0 0 8 2 】

このように、マイクロサテライト・マーカーや S N P 等の遺伝子多型を利用して S H P 1 遺伝子の L O H を確認することで、簡素なメカニズムでより確実に造血器腫瘍細胞を検出することが可能となる。

## 【 0 0 8 3 】

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なお、本実施の形態では、SHP1遺伝子のLOHを、マイクロサテライト・マーカーや遺伝子多型を利用して確認した例を挙げているが、本発明はこれに限定されるものではなく、SHP1遺伝子のLOHが確認できる方法であればどのような方法でもよいことは言うまでもない。

【0084】

次に、本実施の形態にかかる検出方法の好ましい一例について、より具体的に説明する。

【0085】

まず、SHP1遺伝子産物定量工程により、前述した手法を用いて検体試料中に含まれるSHP1蛋白質およびSHP1 mRNAの少なくとも一方を定量する。このプロセスで定量されたSHP1蛋白質が、標準よりも大幅に減少していたり、ほとんどSHP1遺伝子産物が発現していなかったりした場合には、検体試料中に造血器腫瘍細胞が含まれている可能性が高くなる。

【0086】

次に、SHP1遺伝子メチル化確認工程で、前記制限酵素確認法により、検体試料から調製した遺伝子試料中のSHP1遺伝子の塩基配列中に含まれるCpG島のメチル化を確認する。以下の説明では、メチル化感受性制限酵素として前記HpaIIを用いた例を挙げる。HpaIIは、前述したようにCCGGの塩基配列を認識するが、同じ塩基配列を、メチル化非感受性制限酵素MspIも認識するため、好ましく用いられる。

【0087】

そこで、遺伝子切断試行段階では、上記検体試料から得られた遺伝子試料を、HpaIIで処理する。同時に、同一の遺伝子試料をMspIで処理すると好ましい。これによって、CCGG塩基配列が切断されるというポジティブコントロールを得ることができる。

【0088】

次に、遺伝子増幅試行段階に移行するが、このステップでは、先に、SHP1遺伝子の塩基配列（配列番号1および2、図1～図10および図11～図20参照）から、HpaII/MspIの認識部位（CCGG）を挟んでPCR用のプライマーを設定する。具体的には、例えば、後述する実施例1や実施例2に示すプライマーペアを用いる。

【0089】

上記のようなプライマーを用いて、HpaIIで処理された遺伝子試料に対してPCRを実施し、遺伝子増幅量確認段階で、例えば電気泳動によりPCR産物の増幅量を確認する。遺伝子試料中に、メチル化されたSHP1遺伝子があれば、HpaIIは切断できないので、PCRにより目的のサイズのPCR産物が検出できる。一方、メチル化されたSHP1遺伝子が無ければ、HpaIIによりDNAが切断されPCR産物は検出できない。

【0090】

このように、上記制限酵素確認法を用いれば、メチル化感受性制限酵素を用いて検体試料から得られた遺伝子試料に含まれるSHP1遺伝子の切断を試み、さらにPCRを用いて増幅してから、得られるPCR産物の増幅量を確認することができる。それゆえ、検体試料から微量のSHP1遺伝子さえ得られれば、SHP1遺伝子のメチル化を検出することが可能である。そのため、検体試料中に造血器腫瘍細胞がごく微量しか存在していなくても迅速に高い検出感度で、しかも高特異的に造血器腫瘍細胞を検出することが可能となる。

【0091】

なお、本実施の形態で説明した上記検出方法には、他の工程（プロセス）や他の段階（ステップ）が含まれていてもよいことは言うまでも無い。例えば、SHP1遺伝子メチル化確認工程において、制限酵素反応やPCR反応を円滑に進めるために、得られた遺伝子試料等を精製する精製段階が含まれていてもよい。

【0092】

本発明には、上述した造血器腫瘍細胞検出方法だけでなく、該検出方法を実施するための検出キットが含まれる。具体的には、前記SHP1抗体、前記メチル化感受性制限酵素、前記各プライマー、前記SHP1遺伝子陽性およびメチル化陰性対照DNA等を含む構成

を挙げることができる。特に、(1) 上記 S H P 1 抗体、および (2) メチル化感受性制限酵素と、P C R 用プライマーと、前記 S H P 1 遺伝子陽性およびメチル化陰性対照 D N A との組み合わせに分けた場合には、(1) および (2) の少なくとも一方が含まれていると好ましい。また、S H P 1 遺伝子産物定量工程と S H P 1 遺伝子メチル化確認工程の順番はどちらが先であっても良い。

【 0 0 9 3 】

さらに、上記検出キットには、必要に応じて、他の各種試薬類が含まれていてもよい。例えば、ヌクレオチドモノマー、ポリメラーゼ、バッファー等の P C R 反応用試薬、および、バッファー等の制限酵素反応用試薬の少なくとも一方が含まれていてもよい。

【 0 0 9 4 】

より具体的に、各工程または段階ごとに用いられる試薬等について説明する。まず、遺伝子産物定量工程では、蛋白質定量法の場合、酵素抗体法およびウエスタンブロッティング法の何れであっても、S H P 1 抗体およびその検出試薬が少なくとも用いられる。また、m R N A 定量法の場合、R T - P C R 法や r e a l t i m e R T - P C R 法を用いる場合、S H P 1 c D N A 検出用プライマーおよび T a q D N A ポリメラーゼ反応試薬が少なくとも用いられる。

【 0 0 9 5 】

次に、本実施の形態における S H P 1 遺伝子メチル化確認工程では、メチル化感受性制限酵素によりメチル化を確認するため、まず、遺伝子切断試行段階にて、メチル化感受性制限酵素、メチル化非感受性制限酵素、およびこれらの反応試薬が少なくとも用いられる。次に、遺伝子増幅試行段階では、プライマー、T a q D N A ポリメラーゼ反応試薬、システム検討用 S H P 1 遺伝子メチル化陽性 D N A が少なくとも用いられる。次に、遺伝子増幅量確認段階では、S H P 1 遺伝子メチル化陽性およびメチル化陰性対照 D N A を用いた反応産物を電気泳動のコントロールとして少なくとも使用することができる。

【 0 0 9 6 】

このように、本発明にかかる検出キットでは、前述した造血器腫瘍細胞検出方法を実施するために好ましい薬剤や標本等が含まれている。そのため、上記検出キットを用いることで、本発明にかかる造血器腫瘍細胞検出方法を容易かつ簡素に実施することができ、本発明を臨床検査産業や医薬品産業等の産業レベルで利用することが可能となる。

【 0 0 9 7 】

〔実施の形態 2〕

本発明における実施の他の形態について図 2 5 ないし図 4 7 に基づいて説明すれば以下の通りである。なお、本発明はこれに限定されるものではない。また、説明の便宜上、実施の形態 1 と重複する説明は適宜省略する。

【 0 0 9 8 】

前記実施の形態 1 では、S H P 1 遺伝子メチル化確認工程に、メチル化感受性制限酵素を用いる制限酵素確認法を用いたが、本発明は、これに限定されるものではなく、本実施の形態では、例えば、遺伝子修飾段階とメチル化シトシン含有判定段階とを含む、重亜硫酸塩を用いて D N A を修飾する方法（以下、説明の便宜上、D N A 修飾法と称する）を好適に用いることができる。

【 0 0 9 9 】

D N A を重亜硫酸塩 ( B i s u l f i t e ) で処理すると、シトシンはウラシルに変換される。具体的には、図 2 5 に示すように、シトシンが重亜硫酸塩によりスルホン化 ( S u l p h o n a t i o n ) され、さらに加水分解により脱アミノ化 ( H y d r o l y t i c d e a m i n a t i o n ) され、さらに、アルカリ存在下での脱スルホン化 ( A l k a l i d e s u l p h o n a t i o n ) により、ウラシルに変換される。このウラシルは P C R 後、チミンに置き変わる。これに対して、メチル化されたシトシン ( 5 ' - メチルシトシン ) は重亜硫酸塩によって変換されない。そこで、本実施の形態では、この重亜硫酸塩処理後の塩基配列の違いを利用して、後述するように、S H P 1 遺伝子のメチル化の有無を検出する。

## 【 0 1 0 0 】

次に、本実施の形態における S H P 1 遺伝子メチル化確認工程、すなわち D N A 修飾法による S H P 1 遺伝子のメチル化の確認について具体的に説明する。

## 【 0 1 0 1 】

まず、遺伝子修飾段階として、造血器細胞を含む前記検体試料から得られた遺伝子試料を重亜硫酸塩で処理する。この段階では、上述したように、メチル化されていないシトシンのみがウラシルに変換されるので、例えば、それゆえ、D N A を重亜硫酸塩処理すると、図 2 6 に示すように、メチル化された（図中円で囲んだ M で示す）シトシンはシトシンのままで残存するが、メチル化されていないシトシンはウラシル（U）に変換される。

## 【 0 1 0 2 】

上記遺伝子修飾段階で用いられる重亜硫酸塩としては、特に限定されるものではないが、例えば、重亜硫酸ナトリウム（ $\text{Na}_2\text{S}_2\text{O}_5$ 、メタ重亜硫酸ナトリウム、二亜硫酸ナトリウムまたはピロ重亜硫酸ナトリウムともいう）を好適に用いることができる。さらに、重亜硫酸化合物とともに尿素が併用されてもよい。

## 【 0 1 0 3 】

次に、メチル化シトシン含有判定段階として、重亜硫酸塩で処理された遺伝子試料に含まれる、S H P 1 遺伝子の塩基配列中のシトシンの有無を判定する。重亜硫酸塩処理物中の S H P 1 遺伝子にシトシンが含まれているということは、処理前の S H P 1 遺伝子には、メチル化されたシトシンが含まれていることになる。それゆえ、シトシンが存在すれば、元の検体試料中に造血器腫瘍細胞が含まれていることになる。

## 【 0 1 0 4 】

上記メチル化シトシン含有判定段階で実施される、S H P 1 遺伝子の塩基配列中のシトシンの有無を判定する方法としては特に限定されるものではないが、具体的には、1）メチル化シトシンを P C R により検出する方法、2）メチル化シトシンを遺伝子の塩基配列の決定により検出する方法、または、3）メチル化シトシンを含む塩基配列を識別する方法のうち、少なくとも何れかの手法を好ましく用いることができる。

## 【 0 1 0 5 】

より具体的には、まず、1）メチル化シトシンを P C R により検出する方法としては、メチル化特異的 P C R（M e t h y l a t i o n S p e c i f i c P C R）を挙げることができる。

## 【 0 1 0 6 】

上記メチル化特異的 P C R 法は、メチル化された D N A に特異的かつ C G 配列を含む塩基配列をプライマーとして設定する。メチル化されたシトシンが存在していれば P C R により増幅が可能となり、それゆえメチル化された S H P 1 遺伝子を検出することができる。

## 【 0 1 0 7 】

上記メチル化特異的 P C R 法は、従来公知の方法（例えば、P r o c . N a t l . A c a d . S c i . U S A 93, 9821-9826, (1996) 等の文献に開示されている方法）を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。なお、D N A の精製過程ではエタノール沈澱法や G l a s s b e a d s 法を用いた方法等を用いることができ、また、蛍光ラベルしたプライマーを用いれば、P C R の検出を容易にすることができる。

## 【 0 1 0 8 】

次に、2）遺伝子の塩基配列の決定によりメチル化シトシンを検出する方法、すなわち S H P 1 遺伝子のシーケンシングでは、C G 配列を含まない領域にプライマーを設定し P C R を実施する。得られる P C R 産物の中には、メチル化をされているもの（C G 配列のままで存在）とメチル化されていないもの（T G 配列に変換されている）が含まれている可能性がある。これをシーケンシングすることにより、C G 配列すなわちメチル化の存在を検討する。

## 【 0 1 0 9 】

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上記 S H P 1 遺伝子のシークエンシングも、従来公知の方法（例えば、P r o c . N a t l . A c a d . S c i . U S A 89, 1827-1831 (1992) 等の文献に開示されている方法）を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。なお、上記プライマーとしては、メチル化された D N A に特異的な配列（C G 配列を含む）を有するプライマーを用いることも可能である。

#### 【 0 1 1 0 】

この方法も P C R を用いているので、検体試料から微量の S H P 1 遺伝子さえ得られれば、S H P 1 遺伝子のメチル化を検出することが可能である。そのため、検体試料中に造血器腫瘍細胞がごく微量しか存在していなくても高い検出感度で高特異的に造血器腫瘍細胞を検出することが可能となる。また、シークエンシングを利用することにより具体的な配列を決定するので、メチル化の程度をより明確化することも可能となる。

#### 【 0 1 1 1 】

次に、3) シトシンを含む塩基配列を識別する方法としては、M s - S n u P E 法、重亜硫酸塩 S S C P 法、メチルライト法、蛍光溶解曲線分析法、C O B R A 法等を挙げることができる。

#### 【 0 1 1 2 】

上記 M s - S n u P E ( M e t h y l a t i o n - s e n s i t i v e S i n g l e N u c l e o t i d e P r i m e r E x t e n s i o n ) 法は、メチル化された D N A に特異的なプライマーを用いて P C R を実施する方法である。ただし、プライマーに挟まれた領域でのメチル化の有無が判らないので、検出したい C G 配列に隣接するポリヌクレオチドを作成し P C R 産物とアニールさせる。放射性同位元素の存在下で D N A を合成した時に、 $^{32}\text{P}$ -d C T P を取り込めば、そこは C G 配列であるためメチル化されているシトシンが存在することになる。一方、D N A を合成した時に、 $^{32}\text{P}$ -d T T P を取り込めば、そこは T G 配列であるためメチル化はされていないことになる。

#### 【 0 1 1 3 】

上記 M s - S n u P E 法は、従来公知の方法（例えば、N u c l e i c A c i d s R e s e a r c h 25, 2529-2531, (1997) 等の文献に開示されている方法）を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。

#### 【 0 1 1 4 】

上記重亜硫酸塩 S S C P ( B i s u l f i t e - S S C P ) 法も、メチル化された D N A に特異的なプライマーを用いて P C R を実施する方法であるが、プライマーに挟まれた領域でのメチル化の有無が判らない。そこで、P C R 産物を 1 本鎖 D N A に変性後、S S C P ( S i n g l e S t r a n d C o n f o r m a t i o n a l P o l y m o r p h i s m ) 法を用いて電気泳動し、1 本鎖 D N A の移動度の違いから、S H P 1 遺伝子のメチル化の程度を判定する。

#### 【 0 1 1 5 】

上記重亜硫酸塩 S S C P 法も、従来公知の方法（例えば、E l e c t r o p h o r e s i s 21, 904-908, (2000) 等の文献に開示されている方法）を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。

#### 【 0 1 1 6 】

他に、メチルライト ( M e t h y l - l i g h t ) 法や、蛍光溶解曲線分析 ( F l u o r e s c e n c e M e l t i n g C u r v e A n a l y s i s ) 法等も挙げられる。これら方法も、何れもメチル化された D N A に特異的なプライマーを用いて P C R を実施する方法であるが、プライマーに挟まれた領域でのメチル化の有無が判らない。そこで、内側の調べたい領域について、メチル化特異的なポリヌクレオチドを作成し、このメチル化特異的ポリヌクレオチドが 1 本鎖にした P C R 産物とどの程度アニール ( 2 本鎖重合 ) 反応するかを検討することにより、上記 P C R 産物中のメチル化の量を判定する。



## 【 0 1 1 7 】

上記メチルライト法は、具体的には、例えば、N u c l e i c A c i d s R e s e a r c h 28 ( 8 ) , E 3 2 , ( 2 0 0 0 ) 等の文献に開示されている方法を、上記蛍光溶解曲線分析は、具体的には、例えば、C l i n i c a l C h e m i s t r y 47 , 1 1 8 3 - 1 1 8 9 , ( 2 0 0 1 ) 等の文献に開示されている方法を好適に用いることができる。

## 【 0 1 1 8 】

上述した各方法は、P C Rを用いているので、検体試料から微量のS H P 1遺伝子さえ得られれば、S H P 1遺伝子のメチル化を検出することが可能である。そのため、検体試料中に造血器腫瘍細胞がごく微量しか存在していなくても高い検出感度で高特異的に造血器腫瘍細胞を検出することが可能となる。

## 【 0 1 1 9 】

上記C O B R A法 ( C o m b i n e d B i s u l f i t e R e s t r i c t i o n A n a l y s i s、あるいは、B i s u l f i t e P C R f o l l o w e d b y r e s t r i c t i o n a n a l y s i s等とも称される) では、例えば、C G C G配列がメチル化を受けていると、重亜硫酸処理後もC G C G配列のままに残存するが、メチル化されていないとT G T G配列に変換される。そこで、上記C G C G配列のみを切断する制限酵素等を利用することで、電気泳動ゲル上のバンドパターンを解析して、S H P 1遺伝子のメチル化の有無を判定および定量化することができる。

## 【 0 1 2 0 】

上記C O B R A法も、従来公知の方法 (例えば、N u c l e i c A c i d s R e s e a r c h 25 , 2 5 3 2 - 2 5 3 4 , ( 1 9 9 7 ) 等の文献に開示されている方法) を好適に用いることができ、その具体的な工程や試薬類、条件等は特に限定されるものではない。勿論、この方法でもP C Rが用いられるので、上述したP C Rによる利点を得られるだけでなく、制限酵素処理と電気泳動とを用いるので、バンドパターンの解析さえ明確化しておれば、容易にS H P 1遺伝子のメチル化を確認することができるという利点もある。

## 【 0 1 2 1 】

このように、本実施の形態におけるD N A修飾法では、メチル化シトシン含有判定段階でP C Rを用いているが、このP C Rで用いるプライマーの設計方法について以下に説明する。

## 【 0 1 2 2 】

上述したように、D N Aを重亜硫酸塩処理するとシトシンはウラシルに変換されるが、メチル化されたシトシンは変換されずに保存される。ここで、細胞内でメチル化を受ける可能性のあるシトシンは、5'配列側からC Gと並ぶC G配列 ( 5' - C G - 3' ) のシトシン ( C ) のみである。そのため、重亜硫酸塩処理により、上記C G配列以外のシトシンは全てチミン ( T ) に変換されてしまう。そこで、全てのC G配列がメチル化を受けたものとしてS H P 1遺伝子の塩基配列を変換し、プライマーを設定する。なお、D N A中のウラシルはチミンとして認識され、P C Rによりチミンに置換されることになる。

## 【 0 1 2 3 】

まず、プライマーを計画するD N A鎖に関する条件を設定する。S H P 1遺伝子の塩基配列において、センス鎖またはアンチセンス鎖の何れも、上記C G配列のみがメチル化を受けたとして、その他の塩基配列におけるシトシンが全てチミンに変換された配列を想定する。

## 【 0 1 2 4 】

具体的には、図27～図36および配列番号5に示す塩基配列が、図1～図10および配列番号1に示すS H P 1遺伝子のゲノムD N A ( ワイルドタイプ ) のセンス鎖に対応する、重亜硫酸塩処理後の塩基配列 ( 以下、説明の便宜上、センス鎖変換配列とする ) であり、図37～図46および配列番号6に示す塩基配列が、図11～図20および配列番号2に示すS H P 1遺伝子のゲノムD N A ( ワイルドタイプ ) のアンチセンス鎖と対応

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する、重亜硫酸塩処理後の塩基配列（以下、説明の便宜上、アンチセンス鎖変換配列とする）である。これらセンス鎖変換配列とアンチセンス鎖変換配列とは、重亜硫酸塩処理により相補的ではなくなる。

【 0 1 2 5 】

なお、図 2 7 ～ 図 3 6 および配列番号 5、並びに、図 3 7 ～ 図 4 6 および配列番号 6 の塩基配列は、C G 配列が 1 0 0 % メチル化されていると想定した場合に、重亜硫酸塩処理を受けたものとしての塩基配列であり、実際には細胞中で 1 0 0 % のメチル化が生じるとは考えられないため、本発明において検出し得る可能性としての塩基配列として例示する。

【 0 1 2 6 】

そして、( I ) 上記センス鎖変換配列に対して、フォワードプライマー ( F W プライマー ) およびリバースプライマー ( R V プライマー ) を作成するか、あるいは、( I I ) 上記アンチセンス鎖変換配列に対して、F W プライマーおよび R V プライマーを作成する。この場合、同じ場所でもプライマー配列はそれぞれ異なる。 10

【 0 1 2 7 】

次に、プライマーを計画する領域に関する条件を設定する。( i ) メチル化された D N A のみを直接 P C R で増幅するために、C G 配列を含む塩基配列に対してプライマーを作成するか、( i i ) メチル化されたもの、されていないものを区別なく P C R で増幅するために、C G 領域を含まない配列に対してプライマーを作成する。なお、( i i ) の場合は、後でシーケンシングかその他の方法を実施し、メチル化を判定する。

【 0 1 2 8 】

したがって、D N A 修飾法で用いられるプライマーの設計には、上記 D N A 鎖に関する条件 ( I ) および条件 ( I I ) と、領域に関する条件 ( i ) および条件 ( i i ) とを掛け合わせた 4 通りの設計方法がある。 20

【 0 1 2 9 】

ここで、( i ) の場合、プライマーの場所が都合良くメチル化を受けていれば検出されるが、その場所ではなく近隣領域のみメチル化を受けているような場合には、メチル化が存在するのにも関わらず検出不可能となる。そこで、( i i ) のように、メチル化の有無に関わらず P C R で増幅後、各プライマーに囲まれた領域内のメチル化、すなわち C G 配列の有無を検定することで、確実に S H P 1 遺伝子のメチル化を検出することができる。そのため、本実施の形態における S H P 1 遺伝子のメチル化の判定には、検出用のプライマーの場所のみならず、遺伝子配列の情報そのものが重要となる。 30

【 0 1 3 0 】

また、C G 配列がメチル化されていないと、重亜硫酸塩処理により T G 配列に変換されるが、この T G 配列を含む塩基配列に対して作成されるプライマー ( U n m e t h y l a t e d p r i m e r ) は、メチル化を受けていない D N A の存在を証明するコントロールとして用いることができる。また、重亜硫酸塩処理が不十分な場合には、シトシンがウラシルに変換されていないワイルドタイプの S H P 1 遺伝子が混入することになる。そこで、重亜硫酸塩処理が十分完全になされたか否かのコントロールとして、ワイルドタイプの塩基配列を有するプライマー ( W i l d t y p e p r i m e r ) を用いることができる。 40

【 0 1 3 1 】

なお、上述したメチル化シトシン含有判定段階では、P C R により増幅された遺伝子の確認に、前記実施の形態 1 における遺伝子増幅量確認段階と同様の方法、例えば、電気泳動法を用いてマーカと比較することにより遺伝子の増幅量を確認したり、さらに電気泳動後に得られた D N A バンドをメンブレンにブロッティングしたりする手法が挙げられる。勿論、これら手法に限定されるものではなく、また、上記電気泳動法やブロッティングの方法についても従来公知の手法を好適に用いることができ、特に限定されるものではない。

【 0 1 3 2 】

換言すれば、本実施の形態における D N A 修飾法による S H P 1 遺伝子メチル化確認工程 50

でも、前記実施の形態 1 における制限酵素確認法による場合と同様、遺伝子増幅量確認段階が含まれていても良い。

【 0 1 3 3 】

次に、本実施の形態にかかる検出方法の好ましい一例について、より具体的に説明する。

【 0 1 3 4 】

まず、SHP1 遺伝子産物定量工程により、前述した手法を用いて検体試料中に含まれる SHP1 蛋白質および SHP1 mRNA の少なくとも一方を定量する。このプロセスで定量された SHP1 蛋白質が、標準よりも大幅に減少していたり、ほとんど SHP1 遺伝子産物が発現していなかったりした場合には、検体試料中に造血器腫瘍細胞が含まれている可能性が高くなる。

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【 0 1 3 5 】

次に、SHP1 遺伝子メチル化確認工程で、前記 DNA 修飾法により、検体試料から調製した遺伝子試料中の SHP1 遺伝子の塩基配列中に含まれる CpG 島のメチル化を確認する。具体的には、遺伝子修飾段階にて、例えば重亜硫酸ナトリウムを用いて、上記検体試料から得られた遺伝子試料をで処理する。

【 0 1 3 6 】

次に、遺伝子増幅試行段階に移行するが、このステップでは、前述したプライマーの設計方法に基づいて、PCR 用のプライマーを設定する。

【 0 1 3 7 】

具体的には、メチル化特異的 PCR では、図 4 7 ( a ) に示すように、例えば、2 3 塩基対のワイルドタイプ DNA ( 図中上がセンス鎖で下がアンチセンス鎖 ) を想定し、ワイルドタイプ DNA の CG 配列に 1 0 0 % メチル化があるとする。この場合、重亜硫酸塩処理すると、図 4 7 ( b ) に示すように、センス鎖とアンチセンス鎖は相補的ではなくなる。そこで、図 4 7 ( c ) または ( d ) に示すように、センス鎖またはアンチセンス鎖に対して FW プライマーおよび RV プライマーを作成する。

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【 0 1 3 8 】

なお、上記メチル化特定 PCR においては、PCR 用プライマーとして、具体的には、例えば、後述する実施例 4 や実施例 5 に示すプライマーペアを用いる。上記のようなプライマーを用いて、重亜硫酸ナトリウムで処理された遺伝子試料に対してメチル化特異的 PCR を実施し、例えば電気泳動により PCR 産物の増幅量を確認する。

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【 0 1 3 9 】

このように、上記 DNA 修飾法を用いれば、重亜硫酸塩を用いて検体試料から得られた遺伝子試料を処理すると、塩基配列中のシトシンはウラシルに変換されるが、メチル化されたシトシンは変換されない。そのため、遺伝子修飾段階後の SHP1 遺伝子の塩基配列中にシトシンが含まれるか否かを判定するのみで、SHP1 遺伝子のメチル化を検出することができる。そのため、単純なメカニズムで迅速かつ高特異的に造血器腫瘍細胞を検出することが可能となる。

【 0 1 4 0 】

次に、SHP1 遺伝子産物定量工程により、前述した手法を用いて検体試料中に含まれる SHP1 蛋白質および SHP1 mRNA の少なくとも一方を定量する。このプロセスで定量された SHP1 遺伝子産物が、標準よりも大幅に減少していたり、ほとんど発現していなかったりした場合には、検体試料中に造血器腫瘍細胞が含まれている可能性が高くなる。

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【 0 1 4 1 】

なお、本実施の形態で説明した上記検出方法には、前記実施の形態 1 の検出方法と同様に、他の工程 ( プロセス ) や他の段階 ( ステップ ) が含まれていてもよいことは言うまでも無い。

【 0 1 4 2 】

本発明には、上述した造血器腫瘍細胞検出方法だけでなく、該検出方法を実施するための検出キットが含まれる。具体的には、遺伝子処理レベルまで精製された重亜硫酸塩と前記

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プライマー、および前記SHP1抗体を含む構成を挙げることができる。つまり、本発明にかかる検出キットでは、上記重亜硫酸塩、プライマー、およびSHP1抗体を、(1) 上記SHP1抗体、(2) 重亜硫酸塩と、該重亜硫酸塩で処理された遺伝子試料に含まれるSHP1遺伝子の塩基配列中のシトシンの有無の判定用プライマー、および(3) 配列番号3に示すSHP1遺伝子cDNAの塩基配列の全長またはその一部を検出するPCR用のプライマーに分けた場合、(1)、(2)および(3)のうち、少なくとも何れか一つを含むことが好ましい。

#### 【0143】

さらに、上記検出キットには、配列番号3に示すSHP1遺伝子cDNAの塩基配列の全長またはその一部と相同性を持つノーザンブロットング用プローブ、または、シトシンを含む塩基配列を認識する制限酵素およびSHP1遺伝子のメチル化陽性及びメチル化陰性対照DNAを用いた電気泳動用マーカーが含まれていてもよく、さらには、ヌクレオチドモノマー、ポリメラーゼ、バッファー等のPCR反应用試薬、および、バッファー等の制限酵素反应用試薬の少なくとも一方が含まれていてもよい。

#### 【0144】

より具体的に、各工程または段階ごとに用いられる試薬等について説明する。まず、遺伝子産物定量工程では、前記実施の形態1で例に挙げたものと同様であるのでその説明は省略する。

#### 【0145】

次に、本実施の形態におけるSHP1遺伝子メチル化確認工程では、重亜硫酸塩処理によりメチル化を確認するため、まず、遺伝子修飾段階にて、各種重亜硫酸塩等の試薬が少なくとも用いられる。次に、メチル化シトシン含有判定段階では、メチル化シトシンをPCRにより検出する方法を用いる場合には、メチル化配列特異的プライマー、およびTaq DNAポリメラーゼ反応試薬が少なくとも用いられる。また、遺伝子の塩基配列の決定によりメチル化シトシンを検出する方法、あるいはシトシンを含む塩基配列を認識する方法では、各具体的な方法に応じて公知の試薬類を用いる。

#### 【0146】

このように、本実施の形態にかかる検出キットでも、前記実施の形態1の検出キットと同様、前述した造血器腫瘍細胞検出方法を実施するために好ましい薬剤や標本等が含まれている。そのため、上記検出キットを用いることで、本発明にかかる造血器腫瘍細胞検出方法を容易かつ簡素に実施することができ、本発明を臨床検査産業や医薬品産業等の産業レベルで利用することが可能となる。

#### 【0147】

なお、本発明は、上述した各実施の形態に限定されるものではなく、請求項に示した範囲で種々の変更が可能であり、異なる実施の形態にそれぞれ開示された技術的手段を適宜組み合わせて得られる実施の形態についても、本発明の技術的範囲に含まれることはいうまでもない。

#### 【0148】

##### 【実施例】

以下、図48ないし図52に基づいて、本発明の具体的な実施例について説明する。なお、本発明はこれに限定されるものではない。

#### 【0149】

##### 【実施例1】

ナチュラルキラー細胞リンパ腫を含む検体試料を用い、Towbin H. et al : Proc. Natl. Acad. Sci. USA 76, 4350, (1979)に開示されている方法にしたがってウエスタンブロットングを実施した。なお、SHP1抗体として#SH-PTP1(D-11):sc7289(Santa Cruz Biotechnology Inc. 製)を用いた(SHP1遺伝子産物定量工程・蛋白質定量法)。

#### 【0150】

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その後、SHP1 遺伝子メチル化確認工程に移行した。まず、メチル化感受性制限酵素としてHpaIIを用いて、上記検体試料から調製した遺伝子試料を37℃4時間で処理した（遺伝子切断試行段階）。

【0151】

次に、HpaIIで処理した遺伝子試料をPCRで増幅した（遺伝子増幅試行段階）。このとき用いたプライマーペアは、配列番号7および図48（a）に示す19塩基のプライマーREP-S1と、配列番号8および図48（b）に示す20塩基のプライマーREP-AS1との組み合わせとした。このプライマーペアを用いた場合、配列番号9および図48（c）に示すように、SHP1 遺伝子のセンス鎖の配列（配列番号1および図1～図10参照）における、7441塩基から7566塩基までの126塩基の塩基配列が検出される。

【0152】

なお、図48（c）におけるカッコ内の「#（番号）」は、上記SHP1 遺伝子のセンス鎖における塩基の位置を示しており、下線部はプライマーREP-S1およびREP-AS1の対応位置、並びにHpaIIの認識切断部位の位置を示している。また、プライマーREP-AS1は、上記REP-AS1の下線部の領域におけるアンチセンス鎖の配列に対してデザインされたものである。

【0153】

その後、アガロースゲルで電気泳動してから、得られたDNAバンドをナイロンメンブレンにブロッティングしてSHP1 遺伝子の増幅を確認した（遺伝子増幅量確認工程）。

【0154】

次に、Towbin H. et al: Proc. Natl. Acad. Sci. U S A 76, 4350, (1979)に開示されている方法にしたがってウエスタンブロッティングを実施した。なお、SHP1 抗体として#SH-P TP1 (D-11): sc 7289 (Santa Cruz Biotechnology Inc. 製)を用いた（SHP1 遺伝子産物定量工程・蛋白質定量法）。

【0155】

上記SHP1 遺伝子メチル化確認工程とSHP1 遺伝子産物定量工程との結果から検体試料中の造血器腫瘍細胞を検出した。

【0156】

〔実施例2〕

遺伝子増幅試行段階で、プライマーペアとして、配列番号10および図49（a）に示す21塩基のプライマーREP-S2と、配列番号11および図49（b）に示す21塩基のプライマーREP-AS2との組み合わせを用いた以外は、前記実施例1と同様にして検体試料中の造血器腫瘍細胞の有無を検出した。

【0157】

上記プライマーペアを用いた場合、配列番号12および図49（c）に示すように、SHP1 遺伝子のセンス鎖の配列（配列番号1および図1～図10参照）における、6858塩基から7084塩基までの227塩基の塩基配列を検出することができる。

【0158】

なお、図49（c）におけるカッコ内の「#（番号）」も、上記SHP1 遺伝子のセンス鎖における塩基の位置を示しており、下線部はプライマーREP-S2およびREP-AS2の対応位置、並びにHpaIIの認識切断部位の位置を示している。また、プライマーREP-AS2は、上記REP-AS2の下線部の領域におけるアンチセンス鎖の配列に対してデザインされたものである。

【0159】

〔実施例3〕

RT-PCRによるmRNA定量法を用いてSHP1 遺伝子産物定量工程を実施した以外は、前記実施例1と同様にして検体試料中の造血器腫瘍細胞の有無を検討した。

【0160】

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すなわち、前記検体試料から全細胞内のRNAを調製してから逆転写酵素により逆転写した。その後、SHP1特異的プライマーペアを用いてPCRにより増幅した。上記SHP1特異的プライマーペアとしては、配列番号13および図50(a)に示す23塩基のプライマーSHP-PF1と、配列番号14および図50(b)に示す25塩基のプライマーSHP-PR1との組み合わせを用いた。

【0161】

〔実施例4〕

real time RT-PCRによるmRNA定量法を用いてSHP1遺伝子産物定量工程を実施した以外は、前記実施例3（すなわち前記実施例1）と同様にして検体試料中の造血器腫瘍細胞の有無を検討した。上記SHP1特異的プライマーペアとしては、配列番号15および図51(a)に示す20塩基のプライマーSHP-LF1と、配列番号16および図51(b)に示す20塩基のプライマーSHP-LR1を用いた。

【0162】

〔実施例5〕

Proc. Natl. Acad. Sci. USA 93, 9821-9826, (1996) に開示されている方法にしたがってメチル化特異的PCRを用いてSHP1遺伝子メチル化確認工程を実施した以外は、前記実施例1と同様にして検体試料中の造血器腫瘍細胞の有無を検討した。なお、重亜硫酸塩としては、重亜硫酸ナトリウムを用いた。

【0163】

また、上記メチル化特異的PCRにおけるプライマーペアとしては、配列番号17および図52(a)に示す24塩基のプライマーMF2と、配列番号18および図52(b)に示す21塩基のプライマーMR2との組み合わせを用いることができる。このプライマーペアを用いた場合、配列番号19および図52(c)に示すように、SHP1遺伝子のセンス鎖の配列（配列番号1および図1～図10参照）における、7037塩基から7195塩基までの159塩基の塩基配列を検出することができる。

【0164】

なお、図52(c)におけるカッコ内の「#（番号）」は、上記SHP1遺伝子のセンス鎖における塩基の位置を示しており、下線部はプライマーMF2およびMR2の対応位置を示している。ただし、上記各プライマーはメチル化されているDNAのみを検出できるように設計されているので、その塩基配列は、上記下線部の塩基配列とは少し異なっている。また、プライマーMR2は、上記MR2の下線部の領域におけるアンチセンス鎖の配列に対してデザインされたものである。

【0165】

〔実施例6〕

検体試料として、診断用の骨髄(BM)検体と、ALL（急性リンパ芽球性白血病）患者の末梢血(PB)検体とを用いた。ALL患者から得られたBM検体は少なくとも70%の比で芽細胞を含んでいた。また、これら検体試料に対する対照試料は、化学療法によって達成された血液学的完全寛解の後に得られた。

【0166】

上記検体試料を用いてマイクロサテライト解析を行った。このときのPCR反応では、5'側のプライマーを、5'-iodoacatamide fluoresceinでラベルし、反応系は、10 pmolのそれぞれのプライマー、40 ngのゲノムDNA、1×PCRバッファー、200 μMのそれぞれのdNTPと、0.5 unitのTaq DNA polymeraseを含む20 μlの系とした。得られたPCR産物は、ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA) にかき、Genescan Analysis software ver 3.7 (Applied Biosystems) で解析を行った。

【0167】

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その結果、図 5 3 ( a ) ・ ( b ) に示すように、D 1 2 S 3 3 6 マーカーおよび D 1 2 S 3 5 6 マーカーによって S H P 1 遺伝子の L O H の有無を確認できることがわかった。本実施例の結果では、これらマーカーのうちテロメア側の D 1 2 S 3 5 6 マーカーでは、有意な結果が得られた 1 9 症例中 1 5 例 ( 7 9 % ) に L O H が認められた。また、セントロメア側の D 1 2 S 3 3 6 マーカーでは、1 6 症例中 6 例 ( 3 8 % ) に L O H が認められた。

#### 【 0 1 6 8 】

上記何れの実施例の結果も、検体試料から十分に造血器腫瘍細胞を検出することができた。それゆえ、本発明は、複数の診断手法を併用しなくても造血器腫瘍細胞を容易かつ迅速に検出することができることがわかった。

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#### 【 0 1 6 9 】

##### 【発明の効果】

以上のように、本発明にかかる造血器腫瘍細胞検出方法は、造血器細胞を含む検体試料に含まれる、造血器細胞に特異的な S H P 1 蛋白質およびその m R N A の少なくとも一方を定量する S H P 1 遺伝子産物定量工程と、上記検体試料から得られる、上記 S H P 1 蛋白質をコードする S H P 1 遺伝子の塩基配列中に含まれる C p G 島のメチル化を確認する S H P 1 遺伝子メチル化確認工程と、上記検体試料に含まれる S H P 1 遺伝子の異型接合性喪失 ( L O H ) の有無を確認する S H P 1 遺伝子 L O H 確認工程とを含む方法である。

#### 【 0 1 7 0 】

また、本発明にかかる造血器腫瘍細胞検出キットの好ましい一例としては、( 1 ) 造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質を抗原とする S H P 1 抗体、および ( 2 ) シトシンを含む塩基配列を認識するメチル化感受性制限酵素と、S H P 1 遺伝子の塩基配列中に含まれ、上記メチル化感受性制限酵素に認識される塩基配列を含む領域を増幅する P C R 用のプライマーと、上記 S H P 1 遺伝子のメチル化陽性及びメチル化陰性対照 D N A とのうち、少なくとも一方を含む構成か、あるいは、( 1 ) 造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 蛋白質を抗原とする S H P 1 抗体、( 2 ) 遺伝子処理レベルまで精製された重亜硫酸塩と、該重亜硫酸塩で処理された遺伝子試料に含まれる S H P 1 遺伝子の塩基配列中のシトシンの有無の判定用プライマー、および ( 3 ) 配列番号 3 に示す S H P 1 遺伝子 c D N A の塩基配列の全長またはその一部を検出する P C R 用のプライマーのうち、少なくとも何れか一つを含む構成か、または、造血器細胞を含む検体試料から造血器腫瘍細胞を検出するために用いられ、造血器細胞に特異的なプロテインチロシンホスファターゼ S H P 1 遺伝子を挟み込む 2 つのマイクロサテライト・マーカーの少なくとも一方の全長またはその一部を検出する P C R 用のプライマーを含む構成を挙げることができる。

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#### 【 0 1 7 1 】

本発明の方法または構成によれば、S H P 1 遺伝子の発現を、遺伝子 D N A の修飾と m R N A と蛋白質と対立遺伝子の喪失という最大で四重のマーカーを用いて判定できることになる。すなわち、S H P 1 遺伝子の発現低下という一つの造血器腫瘍細胞特異的な現象を 4 段階で確認することができるため、非常に高い特異性で造血器腫瘍細胞を検出することができる。よって、本発明を用いることで、造血器細胞を含む微量の検体試料から造血器腫瘍細胞を容易かつ迅速に検出することができる。

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#### 【 0 1 7 2 】

それゆえ、本発明における悪性リンパ腫・白血病の高感度検出法を用いると、一般集団検診による造血器腫瘍の早期発見、診断および治療後のモニタリングや再発の早期発見が可能になり、これら疾患を発症した家族等血縁者における発症危険度の予測等に本発明を利用することも可能となる。その結果、本発明を臨床検査産業や医薬品産業等の産業レベルで利用することが可能となるという効果を奏する。

#### 【 0 1 7 3 】

##### 【配列表】

<110> Japan Science and Technology Corporation

<120> Hematopoietic malignant cell-d  
lignant cell-detection kit

<130> Y2002-P331



&lt;160&gt; 19

&lt;170&gt; PatentIn Ver. 2. 1

&lt;210&gt; 1

&lt;211&gt; 18404

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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&lt;220&gt;

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| c c t g g g c t g a | g g a a a c c t c a | c a a c c t c a c t | t c t | 10 |
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| a a a t c c g t t t | g a a c c c t g g g | c t c c c c t t c a | g t g |    |
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| a g a t t c a t c c | t t a g c t t c t c | t c c t c c a a a t | a t t | 20 |
| c a g a g g t g g g | c t c t g g g t t c | g a a g c c c g g t | t a g |    |
| c t g g g a g g t c | g a g g c t g c a g | a g a g c t g t a a | c c g |    |
| g a g c t c t g g a | a g c t t g c c c t | a g a g t c a g t c | a a g |    |
| g c g t c a g t t t | c c t c a t c t a t | a a a a t g g g g g | t a a |    |
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| a g g t g c t t g a | t t t c c g g c c c | c t c t c t g t g a | a t g |    |
| c t g g g t c t t a | c c t t c c c t g a | c g c t g c c t t c | t c t | 30 |
| g g g c a g g c a g | a g a c g c t g c t | g c a g g c c a a g | g g c |    |
| a g c c t c a g c c | a g c c t g g a g a | c t t c g t g c t t | t c t |    |
| c c a g g c t c c c | c g c t c a g g g t | c a c c c a c a t c | a a g |    |
| g g c g g g g g a g | c c t c t g c t g a | g g c t c c t g t c | t g t |    |
| c t g c c t g g g c | t t g a a t t c a a | g g c t g g g g a c | c c a |    |
| g c c t a a t t t g | g c t c c c c c c a | g g g t g g a c g c | t a c |    |
| a g c c t c a c g g | a c c t g g t g g a | g c a t t t c a a g | a a g | 40 |
| t t t g t c t a c c | t g c g g c a g g t | c a g g g g t g g g | c c c |    |

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| t c a g g c a g a g   | a a t a g g g g a a | t g g g a a c c t g | c c t |    |
| t g g a c c c c a g   | g c c t g c g a c g | g c c t c t g g c t | t c c |    |
| t g g g a c a g g g   | c a a g t c g g c t | g a a t c t a g a g | g t g |    |
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| a a g g a a g t g g   | g t g t g g g g g g | t t a t t t t t g a | c a a |    |
| t c a g g g c a t c   | a g c t t g c t g g | g c t c a g c t g a | g g g |    |
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| g g a a a a g g g a   | a g t g a a g c c a | t g c t g a g a g a | c g c |    |
| g a g g g g c t c a g | g g t a c c t g g g | a g c c g g c a g g | a c a | 20 |
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| c a t g c a g a g c   | t g g g c a a a c c | t c c a t c a t c a | c t t |    |
| c c a t c a c t g g   | a g g c t c a g g c | t g c t c c t g t g | g t g |    |
| c c c c c c t t c c   | c g g g g a g g g c | t t g a c t g g c c | t c t |    |
| c g g c a t c g g c   | c g c a c a g g g a | c c a t c a t t g t | c a t |    |
| c a a g g g t g a g   | g g g c a c c t g g | g g g t t t g g g g | g t g | 30 |
| c t a t g c c t g g   | a c c t g a g g t t | t g a c t g c c c c | c c a |    |
| c c a g a a g a c c   | a t c c a g a t g g | t g c g g g c g c a | g c g |    |
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| a a g g g c c a g g   | a g t c g g a g t a | c g g g a a c a t c | a c c |    |
| g c c a a g g c c t   | c c c g c a c c t c | g t c c a a g t g a | g t g | 40 |
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18404

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&lt;211&gt; 18404

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

10

&lt;220&gt;

&lt;223&gt; genomic DNA

&lt;400&gt; 2

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| gggggctctg  | taaggatttg   | ctgagtaatg  | cac |
| gctgtctcag  | ccttttccagg  | agtgggcgcc  | agc |
| tttactcccc  | aggacaccag   | tgttttcaaat | aaa |
| aaggaagagg  | ggacaaaaaaaa | caaagcactc  | ttc |
| caagaggata  | agggcagcag   | cggcctggat  | gag |
| ctgaggagcc  | tgctccctgtt  | cccgccagag  | ggg |
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| accaccctgt  | tatgatcgcc   | ctccatttta  | aag |
| tcaccctccc  | acgtgcgagg   | cggagtcccg  | acc |
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| cccactcact  | gggcaagcaa   | agggcctcaga | ggc |

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c c c c a g g g c c c   a c c c t c a g c t   g a g c c c a g c a   a g c
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a a a g g g c c a t g   g g g c t t t c a a   g a a g t g t g t g   a a t
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| a c c t g g g c t c | t c g t c t c a g a | t c a a c c a c c a | a c a |
| c c g g t a c a t c | c g t t t g t c a t | t c a a c a a a c g | t t g |
| t g c a a a c c a c | a c a g c t t c a c | a g a c a c a a a t | a a c |
| g a g c t c a c a g | a g a c a g g t g a | c c g g g g g a g a | c a c |
| c g t a a t t c t c | c c t c t a g g t c | c t g g a g g a a a | c a a |
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| t t c c t a g c a g | a a a a t g a g c t | t g t g c a a a g a | a a g |
| c t g g g g g c g g | t g a g c c t t c a | a g a g a a g c g t | g c g |
| a t g c c a g g c c | t t a g a g t g c g | a c t t t a t c c c | a a a |
| c a g a a a g g a a | g g a a c t t g a c | c c a g t t t g t g | t c a |
| a g a g g g t g c t | g t g t a g g g g g | a g c c t g c a g g | g t a |
| g g c a g g g a g g | a c a g g a g g g g | c c t g g a t g c a | g a c |
| g a c a g c c t g g | a a c c t g g g t c | t g g a g c t c a g | a a g |
| g g g t c a c g t t | g t g t a g t c g a | g a c t t t g t c a | c t a |
| c c c t c a c c c c | t g a g a t c c c t | t t c t g c c c t g | g c a |
| g a c c a c a a g c | a c g g g g g c g g | a t g c g g g g a a | g g g |
| c t c t c g g g t g | g t c a t g a c g a | t g a c a c g g c t | g t t |
| a t t g a c c g t g | g c c t c c a g a c | a a c c c t g g c t | g g c |
| g c c t a g c a g c | t g g t t c t g g a | c g c a a g c g t g | g a g |
| t g g c c c a g c a | c a g g c c c t g a | a c c a c t g c c a | c c c |
| t t t g c c c a g c | t g t c t c t g g a | t c t a g g g t c c | c c c |
| g c c t c t c c t c | c c a c g t g g c c | c a c a c t g c t g | a c c |
| g a c c c g g g g a | t g t t a c t g t c | c c g t c c c t g c | a g g |
| g g g c g g a g a g | g a g g c g a g a t | g g t c t g g g t t | a g c |

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|                       |                     |                     |       |    |
|-----------------------|---------------------|---------------------|-------|----|
| t g g t g g g a g a   | t g g a c a c a c a | g a c t c a a g t c | a c a |    |
| a g g g a g g c g g   | g g a a g a c a a c | c c c a c t g g g g | a a a |    |
| c t g g a t g a t g   | t c a c t g a a g g | g g a g c c c a g g | g t t |    |
| c a g a a a g c a g   | a a c t a a g c c c | t t t c c c c g a a | g c a |    |
| c c c c a c a a a g   | c c c a g c c c c a | c a g g a a a c c a | c g t |    |
| g g g a g a g a g t   | g a g a a g t g a g | g t t g t g a g g t | t t c | 10 |
| g c a a a t t t t t c | c a a c c g c a g g | g a t g g g g g g a | a g g |    |
| t c t c a c c c c t   | g c c c g g a g g t | g a c t c c a g c c | g a g |    |
| g g g c t a c t t c   | a g c c c a c c g c | c t g g g g a c a g | g c t |    |
| g a g c c c c t t c   | c c t c a g g c c c | c a g g a t g a g g | a a g |    |
| a g g a a g c g c a   | g a g c c a c c g a | g g g a c a g a t g | c a a |    |
| c a a g g c a g a g   | g a c a c a g c a g | a a c c t g g g a g | a a a |    |
| g g g t g a c a c t   | g g c t c c t g g g | a c g g g t c a c c | c a a | 20 |
| c t t a a c c t c c   | c g c c c c g g c a | c t g c c c t c c c | t t c |    |
| a c c c a g g c c t   | c a g c c t c c t c | t g c a g g g g c c | t c c |    |
| c a g g c a c t g g   | t c a g c c c g t c | c t c c t c t c c t | g g c |    |
| a g c t t t a g a g   | g g t c t c g g t g | c c a a a a g c c c | c c t |    |
| c a g g g g g c a g   | a c a c g g a g c c | a g c c a t g t g a | g g c |    |
| a g g c c g a t c c   | a g g a a a g g a g | g c c t c c c a g g | t g g |    |
| c a g c t c t a a g   | t t t c a a t c a g | t g c c t t g a c c | a a a | 30 |
| t c t c a g a g a g   | g c t g c a a g c t | g g c a g g a g c a | a g g |    |
| t g a a t g c a g g   | g a t c c a c g g c | a a g g g c a a g t | c g g |    |
| c c c t a g c g t t   | a t t c c g c t g c | c c t t t a c t c t | a a g |    |
| g a a a g g a g c c   | a t g g t g t c a t | g a g g a g c a t t | c a g |    |
| g g g a g a c c a c   | a g g a g g a g t c | g g g g a g g c c a | c a g |    |
| t g c c c g g g a c   | a g c g t g a g a c | a c t t t a c a c a | g g t |    |
| t c t c t g t t t g   | g g c c g g a g a c | c t a t t t g a c c | c c a | 40 |
| c t g g g a c a a g   | g c a t c a a g t g | t c t t g g c a a g | c a t |    |

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|             |             |             |     |
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| cgaggcagct  | ttctgtggct  | tcctcgggggt | tag |
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| aggggagggg  | atggagggtta | ggcagcgccg  | gct |
| cagggtggga  | gccggctcat  | ccctgcttgg  | gaa |
| cactagtggg  | atcggagcag  | ttcagcgggt  | act |
| cctgcaggac  | accctgctgc  | tgagtgtagt  | act |
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| gatccccccac | cctgcaggggc | accaggcggt  | gag |
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| ccctatgaaa  | cagggggctat | ctttatcatt  | tta |

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|                       |                       |                       |       |
|-----------------------|-----------------------|-----------------------|-------|
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| c a a t t c t c c t   | g c c t c a g c c t   | c c c t a g t a g c   | t g g |
| g c c t a a t t t t   | t t t t t t g t a t   | c t t t a g t t g a   | g a c |
| t c t t g a a c t c   | c t g a c c t c g t   | g a t c c a c c t g   | c c t |
| g c t t g a g c c a   | c c a t g c c c a g   | c c g g t t g t c a   | c t t |
| c t t t t t c t t t   | t t c t t t c c t t   | t c t t c t t t c g   | a c t |
| t t c t t t t c t t   | t c t t t c c t t c   | c t t c c t t t t c   | t t t |
| c t t c t t t c t c   | t t t t t c t t t c   | t t c c t t c c c t   | t c c |
| t t t t t t t g a c   | a c a g a a t c t t   | g c t t t a t c a c   | c c a |
| t c a c t g c a g c   | g t c g a c c t c c   | t t g g c t c a g g   | t g a |
| t g a g a c c a c a   | g g t g c a t g c c   | a c t a c a t c c a   | g c t |
| t c g t a g t a g a   | g a t g a g g t c t   | c t c c c t a t g t   | t g c |
| a a g t g a t c c t   | c c c a c c t c a g   | c c t c c c a a a c   | t g c |
| t a c c c a g t a t   | t t t t t t t t t a a | t a g a g a t g g g   | g t c |
| a c t c c t g a g c   | t c a a g c a a t c   | t t c c c c c t t c   | g g c |
| g c g c c a c c t c   | a t c t c g t c a g   | g t t t t c a c t t   | t c a |
| t g c t g t g g c c   | c t g c c t c c g c   | t c c a c t a g g g   | t c c |
| t g t t t g t t c c   | c a c a t t a g g g   | c t t t g t g c t c   | a c c |
| c t t g a t c a c t   | a c g t c a c a t g   | g c c a g c t c c t   | c t t |
| g t c a c c t c c t   | c g g g a a g g c c   | t c c c t g a t c c   | c t c |
| g t c a c t t g c t   | a g c a t a t g a a   | g c t g g t t c a t   | t t t |
| g t c t c a c t c t   | g t c g c c c a g g   | c t g g t g t g c a   | g t g |
| a c c t c c c a g g   | c t c a a g c a a g   | c c t c t c a c c t   | t a g |
| t g t g c c a c c a   | c a c c c a g c t a   | a t t t c t t a t t   | t t t |
| c c a g g c t g g t   | c t c a a a c t c c   | t g g g c t c a a g   | c a a |
| t g g g a t c a c a   | g g t g t g a a c c   | a c t a c a c c c g   | g c c |
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| g t g c g g a g a a | g t t g g c t g g a | c g t g g g a g g g | c c t |
| t g a g g t g t t g | t g a g c t a c c c | a g g g g g a c a g | g t c |
| a g a g g c t g g a | a g t a g a a a t g | c a a t g g a g a g | a a c |
| t t a a a g a g g g | g g c a g a g g a g | c a t g a g t g a g | c c g |
| a a g t t c a t a g | g g a c c g g a a a | c a g g c g c a g g | g c a |
| c g t c g g a g t g | a g c a t c a a c a | a a g c c c t c c c | t c c |
| c c g a t g g t g c | c c g g g a c t t a | c c c a c g g g a c | a g c |
| g g c g g a g g g c | c g c a g g g g g c | g g g g c t g a g c | c a g |
| c c a g g t g a g g | a a g a a c c g c t | c a g t a a t c a g | c c a |
| c a a t g g c a c t | g c a g g g a c a a | a c c c t g g a c t | a a t |
| g g g g c t g g c c | c g a g a c c g c c | c a c a g c g g c c | a g c |
| a g c a g g c c c g | c c t g c t g g t c | c a g g g c c g c c | t t c |
| c t c c a c c t g g | a g c t c a a g c t | g a c a g a g c g g | c g g |
| a g t a a c c c t g | g c t g a g g a a g | a g c a a c c a a t | g g g |
| a g g g c c t a a g | g a a a a t c c c t | a a c a g c c c a g | t t c |
| g g c c c a c c c a | c c c a g g g c g c | t g a g g g a c c c | a c t |
| g g g g a g g a g c | c t c a g g t a g g | g t t a g g c c c t | g g g |
| g g g a g g a g c t | g t t c a g g g g g | a t g g g g c c t g | g a a |
| t g g g g g t t g t | c a g g t c c g c a | a g a g a c t g a c | a c c |
| t t g t c t g g a a | c a t c a a g g c c | t t t c c t c c c c | t g g |
| a g g a g g c g g c | c a c g c t g c c a | c c a g c a g c a g | g c c |
| g a a a c a g c g c | c t t c a g c t t g | c c t g a c a g g c | t g g |
| a c t t g a c c a a | g c g a g c a a a c | t t a a g g a c a c | c t g |
| t c c t a a g g a c | c a g c g t g c c t | a a g a c a c c t g | g a g |
| c c c c c a c t g g | c t g g g c a g c t | g t t t g c c a a a | c a g |
| g t t t g g a t g a | t g a c a t t t g g | t c t a c a c t g g | g c a |

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&lt;210&gt; 3

&lt;211&gt; 1794

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; complementary DNA

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&lt;400&gt; 3

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&lt;210&gt; 4

&lt;211&gt; 595

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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&lt;400&gt; 4

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Leu Lys Gly Arg Gly Val His Gly Ser
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Lys Asn Gln Gly Asp Phe Ser Leu Ser
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Gly Glu Lys Phe Ala Thr Leu Thr Glu  
 65 70

Gln Gln Gly Val Leu Gln Asp Arg Asp  
 85

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Tyr Pro Leu Asn Cys Ser Asp Pro Thr  
 100 105

His Met Ser Gly Gly Gln Ala Glu Thr  
 115 120

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Pro Trp Thr Phe Leu Val Arg Glu Ser  
 130 135

Val Leu Ser Val Leu Ser Asp Gln Pro  
 145 150

Leu Arg Val Thr His Ile Lys Val Met  
 165

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Val Gly Gly Leu Glu Thr Phe Asp Ser  
 180 185

Phe Lys Lys Thr Gly Ile Glu Glu Ala  
 195 200

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Arg Gln Pro Tyr Tyr Ala Thr Arg Val Asn Ala Ala Asp Ile Glu Asn  
 210 215

Arg Val Leu Glu Leu Asn Lys Lys Gln  
 225 230

Ala Gly Phe Trp Glu Glu Phe Glu Ser  
 245

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Asn Leu His Gln Arg Leu Glu Gly Gln  
 260 265

Asn Arg Tyr Lys Asn Ile Leu Pro Phe  
 275 280

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Gln Gly Arg Asp Ser Asn Ile Pro Gly  
 290 295

Tyr Ile Lys Asn Gln Leu Leu Gly Pro  
 305 310

Ile Ala Ser Gln Gly Cys Leu Glu Ala  
 325

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Met Ala Trp Gln Glu Asn Ser Arg Val  
 340 345

Val Glu Lys Gly Arg Asn Lys Cys Val  
 355 360 365

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|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gln | Arg | Ala | Tyr | Gly | Pro | Tyr | Ser |
|     | 370 |     |     |     |     | 375 |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Thr | Thr | Glu | Tyr | Lys | Leu | Arg | Thr |
| 385 |     |     |     |     | 390 |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Gly | Asp | Leu | Ile | Arg | Glu | Ile | Trp |
|     |     |     |     | 405 |     |     |     |     |

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|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Asp | His | Gly | Val | Pro | Ser | Glu | Pro |
|     |     |     | 420 |     |     |     |     | 425 |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Asp | Gln | Ile | Asn | Gln | Arg | Gln | Glu |
|     |     | 435 |     |     |     |     | 440 |     |

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|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Ile | Val | His | Cys | Ser | Ala | Gly | Ile |
|     | 450 |     |     |     |     | 455 |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ile | Asp | Met | Leu | Met | Glu | Asn | Ile |
| 465 |     |     |     |     | 470 |     |     |     |

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|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ile | Asp | Ile | Gln | Lys | Thr | Ile | Gln |
|     |     |     |     | 485 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Met | Val | Gln | Thr | Glu | Ala | Gln | Tyr |
|     |     |     | 500 |     |     |     |     | 505 |

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<400> 5  
t t a t t t a g t t g t g t t t a g t g t a g a t t a a a t g t t

ttgtatgcgt ttgttttggg aaatagttgt ttagttagt ggggagtagt ttagtttag 120

|                     |                       |                     |       |
|---------------------|-----------------------|---------------------|-------|
| a t t a t t a t t t | t t t t t a g g t g   | t t t t a g g t a c | g t t |
| a t t t t t t t t t | g t a g g t g t t t   | t t a a g t t t g t | t c g |
| t t t t g a g a t c | g t t a g t t t g t   | t a g g t a a g t t | g a a |
| g t t a t t t t a t | g g g t t t g t t g   | t t g g t g g t a g | c g t |
| t t g t g t t a t t | g t t a g g g g g a g | g a a a g g t t t t | g a t |
| g a t t t a g t t t | t g g t g t t a g t   | t t t t t g c g g a | t t t |
| t t t t t t t g t t | t t t t t a g g t t   | t t a t t t t t t t | g a a |
| t t t a a t t t t g | t t t t a g g g t t   | t a a t t t t a t t | t g a |
| t g a g a g g t t g | g a g t g g g t t t   | t t t a g c g t t t | t g g |
| t t t t t t t t g a | g g a a t t g g g t   | t g t t a g g g a t | t t t |
| g a g a g g t t t t | t t t t a t t g g t   | t g t t t t t t t t | t a g |
| t a t t t a a t a t | t t c g t c g t t t   | t g t t a g t t t g | a g t |
| t t t t t t c g g g | g g a a g g c g g t   | t t t g g a t t a g | t a g |
| g t t g t a g g g a | a g t t g g t c g t   | t g t g g g c g g t | t t c |
| t t t t g g a g a t | t a t t a g t t t a   | g g g t t t g t t t | t t g |
| c g a g g a g g a a | g t g g t t g a t t   | a t t g a g c g g t | t t t |
| t a g t t g t g t c | g t t g g t t t a g   | t t t c g t t t t t | t g c |
| t a t a g a g a g a | t g t t g t t t c g   | t g g g t a a g t t | t c g |
| t a g t t t t g g a | g g g a g g g a g g   | g t t t t g t t g a | t g t |
| c g a t t t g t c g | t t g t t t t g c g   | t t t g t t t t c g | g t t |
| g t g a g g a t t t | t c g g t t t a t t   | t a t g t t t t t t | t g t |
| a g t g t g t a t t | t g t t t t t t t t   | a t t g t a t t t t | t a t |
| t t t t t g t t t a | g g a t t t g t t t   | t t t t g g g t a g | t t t |
| g t t a t t c g c g | a a g g t t t t t t   | t a c g t t t a g t | t a a |
| t t g g t t t t a t | t t t t t t t g t t   | t t t t t t t a t t | t t t |
| g t a c g t g t t g | a g t a t t t a t t   | a t g t a t t a g g | t g t |
| g a a t a a g a t a | g a t a t g g t t t   | t t g t t t t t a c | g g a |
| t c g a a t a a a t | a a t t t a a t a a   | a t t g g a t t a t | t g t |

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tagatagittt tggicgggtg tagtggittt tatttgtgat tttagtattg tgggaggttg 1860

|              |               |              |     |    |
|--------------|---------------|--------------|-----|----|
| aggcgagagg   | attgttttgag   | tttagggagtt  | tga |    |
| ttgttttttat  | aaaaaaaaataag | aaatttagttg  | ggt |    |
| ttatggagag   | gttaagggtga   | gaggttttgtt  | tga |    |
| gatgattgta   | ttattgtata    | ttagttttggg  | cga |    |
| aaaaaaaaaaaa | gaaaaatgaat   | tagtttttata  | tgt |    |
| attatttagtt  | ggaggggatta   | gggagggtttt  | ttc | 10 |
| ggatgaggag   | gaagaggaggt   | tggttatatgtg | acg |    |
| gtagaggaga   | tggtgagtata   | aaagtttttaa  | tgt |    |
| ttcgtggtag   | aggatttttag   | tggagcggag   | gta |    |
| agttaggatg   | ttgaaagtga    | aaattttgacg  | aga |    |
| tatttttggga  | ggtcgaagggg   | ggaagatttgt  | ttg |    |
| taatatagag   | agatttttatt   | tttatttaaaa  | aaa |    |
| tggtagttttt  | agtagttttgg   | gaggtttgagg  | tgg | 20 |
| gattatttttg  | ggtaatatag    | ggagagatttt  | tat |    |
| tatttaataaaa | tagtttggaatg  | tagtggtatg   | tat |    |
| aggtaggagg   | attatttttgag  | ttaaggagggt  | cga |    |
| gtatttttagt  | ttggggtgata   | aagtaagattt  | ttg |    |
| gaaggaaaga   | aggaaggggaa   | ggaagaaaga   | aaa |    |
| cgagaaagaa   | gaaagaaaaag   | gaagggaaggga | aag |    |
| aagtgatattt  | tagtcgaaag    | aagaaaggga   | aga | 30 |
| gaaaaaaagaa  | aaagtgataa    | tcggtttgggt  | atg |    |
| ttgggagggtc  | gaggtaggtg    | gattacgagg   | tta |    |
| ggtgaaatttt  | tgtttttaatt   | aaagatatata  | aaa |    |
| attttgtgagt  | tttagtttatt   | agggagggttg  | agg |    |
| ggaggtttgta  | gtgagtcgag    | attgcgtttat  | tgt |    |
| ttttattttta  | aaaaaaaaaaaa  | aaaaagaaaaa  | gaa |    |
| ggcgagttttg  | tgggtgggtg    | gttttttttagt | ttt | 40 |
| ttgttttttgt  | tttagtgtat    | atttttgtttat | tgt |    |

gttttttgggt ttttgatttc gtttgigggtt atttttttgt taggtagitt ggtaggitt 3600

|                       |                       |                       |       |
|-----------------------|-----------------------|-----------------------|-------|
| t t t t t g g t g t   | a g a t t t t a t t   | t t t g g t t t t t   | t a g |
| g g g t t t t t a t   | t t t t t a g a a t   | a a t t t t g t t t   | t a g |
| t g t t t a t g t g   | g a t t t t g t g c   | g t g t t a t t t t   | t t t |
| g t a t t t t t t t   | t t t t a t t t t t   | t a t t a t g g g t   | t g t |
| c g t t g t t t t g t | t g t a g t a t g g   | t t g t t g g g g g   | a a a |
| c g t g t t t t a t   | t t a t t t t t t t   | a t t t a t t a g t   | t t g |
| t g a g t t t t a g   | t t t t t g t t t t   | t a a a a t t g g g   | t g a |
| g a g g a t t a a t   | a g t a t a a t g t   | a a a a g t t g g t   | a g t |
| t t a t a t t a g t   | a t t t g g g a a a   | t a t t g t t a a g   | t t t |
| a g a g t a g t t t   | t a g a a t t t t t   | t a t a g a t t a t   | t t t |
| t t t t t t t t g t a | t t a t t a t t g a   | t t t t g a t t t g   | t a t |
| g g a g t t t t a t   | t t t g t t g t t t   | a g g t t g g a g t   | g c g |
| t t t a t t t t t t t | g a g a a g t t g g   | g a t t a t a g g t   | t a g |
| a g t t g g t t t c   | g a a t t t t t t g a | t t t t a a g t g a   | t t t |
| g a t t a t a g g t   | g t a a g t t a t t   | g c g t t t a g t t   | g t a |
| t t t g t t a t t t   | a g g t c g g a t t   | a t a g t g g t a t   | a a t |
| t a g g t t t a a g   | c g a t t t t t t t   | a t t t t a g t t t   | t t t |
| a t t a t a t t t t g | g t t a a a t t t t   | g t a t t t t t t t g | t a g |
| t t g t t t t t t a a | a t t t g g t g t t   | a a g t a a t t t t a | t t a |
| a t a g g c g t g a   | g t t a t t g c g t   | t t g g t t t t t g a | t t t |
| a a t a t t t t t t t | t t t t t t t g a a t | t a t t a g g t a t   | t t a |
| t t g t t t t t t g t | t t a t a a g a a a   | a t g g g g a a a a   | t g a |
| t t a a a t g a g a   | t c g t g t a t g t   | g a a a g t g a t t   | t g t |
| a g g t a g t t t t   | t t a t t t t t t t   | g t t a a a g g a t   | a g t |
| g t t t t t t t g g t | t t g a t a t t t t   | t t a g a g g a a t   | t t t |
| g t t t a g t t a g   | t t t t t t t t t g g | g t g t c g a g t t   | a a t |
| t g g a a g g a g t   | g t t t t g g g g t   | t a g g g t g t a g   | t g g |
| a t t t c g t g t g   | t g g t t a g a t t   | t a t g t t t t a t   | g c g |

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gtgtaggtag atatggatga tgagggtgtgt gtgtgtgtgt gtgtgtgtgt ttttgtttg 5340

|              |              |              |     |
|--------------|--------------|--------------|-----|
| cgtatgataa   | gtagggttgtg  | tgtgtaggat   | tag |
| gttataggatt  | gacgagttttg  | tttggttgaa   | att |
| tttgaatat    | tcgagatgag   | cgagagcggt   | agc |
| tatgtgtttt   | ttttgttttt   | tggcggttggg  | cgt |
| gggggtgggtgt | ttaagtttgaa  | gggggtagtta  | aag |
| agattttttta  | gggtatttagt  | gagagaagaa   | aat |
| aagaaaagtg   | gaaaagttttt  | tttttgggggga | aaa |
| ataatgtagt   | tattatggga   | aaatttagatt  | tgt |
| ttgaagtaga   | atatgtataa   | tgtttataaaa  | tat |
| ttttttttttt  | gaaatagagt   | tttatttgtgt  | tgt |
| ggtttcgttgt  | aatttttgggt  | tttttggggttt | aag |
| agttggggatt  | ataggcggttt  | attatttatgt  | tta |
| aggggggtttt  | attatgtttgg  | ttaggatatgg  | ttt |
| tttgggttttt  | taaagtgtttg  | ggattatagg   | cgt |
| tttataataa   | tttatgtataa  | tagtattttag  | ata |
| aaagttttttt  | agaaaattggt  | tttaggttagt  | ata |
| tattataataa  | tattttaaaaa  | ggtatgtaga   | gaa |
| tagttatttta  | gtttttttttt  | ttagggggaag  | tta |
| gttgagtttgt  | tttttttttcgt | tttgggttttg  | cgg |
| gtaggtagta   | ttataataatt  | tagtggttttag | ggt |
| ttagttatagg  | tgaggattttt  | gttttttttagt | ttt |
| ttgggggtagg  | gtagagggttt  | agggataaga   | att |
| ttaggggttat  | gttggttttat  | ttttttgtttat | agt |
| agaggagggt   | atttttttttt  | tttgtaagta   | ttg |
| ttttatggta   | gttttttttttg | ataaggagggt  | ttt |
| gttggtgtat   | aggagtttta   | agtattgggt   | ttg |
| ggtattgtag   | ttgattttatt  | gatggatttta  | ggt |
| tttttttgttt  | gtaaaaatgat  | aaagatatgtt  | ttt |

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attagataag gtatgtgaac gttattatag tatagcgttc ggtatttagt aggatttatt 7080

|                     |                       |                       |       |
|---------------------|-----------------------|-----------------------|-------|
| c g a t g a t a g t | t g t t a t c g t t   | a t t a t t g t t a   | t t a |
| g t a g t t g g t g | g a g g a g g g a g   | a g a t g t c g t g   | g g a |
| t a t t t g g g t t | t g g a g t g t g t   | a a g g t a t a t a   | t g t |
| g t g t a a t g t t | a t g t t t t t g a   | g t t t t t g a t t   | g t a |
| a t t t t t a g t g | t t a t t t t t g t t | t t g t t t t t t t t | t t t |
| a a g t g a g t t t | t t t t a a g g g g   | t c g g t c g c g t   | t t t |
| t a g g t t a g t g | g a g t g g t a g t   | t t t a g a a t t g   | g g a |
| a t t g g g a g t t | g t a t t t t g a g g | t t t a g t t t t t t | g a g |
| t t t t t t a t t t | t t t g c g t t t t   | t t t t t t t t t t c | g g a |
| t g t t a t t t a c | g g t a g a t a g g   | a g g t a a g g g t   | g t t |
| t t t t g t t t g g | g t c g t t t a g g   | t g g t t t t a t c   | g a g |
| t g t t t a a g g g | t c g a g g t g t t   | t a c g g t a g t t   | t t t |
| g t g a t t t t t c | g t t t t t t c g t t | a g g t a g g t g g   | g t t |
| t t t t t t g t g t | t a t t t a g g t t   | t t g a a t t a t t   | t a t |
| t t t c g t t t g t | t t t t t t g t t t   | t t a a t t t t t a   | t a t |
| g t t t a t g t g t | g t t t t t a t t t   | a g g a t t t t a g   | t c g |
| t a t c g a t t g g | t t t t a t c g t t   | t g g t g t t t t g   | t a g |
| g a t t t a g a a t | t t a g g g g a t t   | t t t a t g a t t t   | g t a |
| a g a g t t g g t g | g a g t a t t a t a   | t t t a g t a g t a   | g g g |
| t a t t t a t t t t | a a g t a t t c g t   | t g a a t t g t t t   | c g a |
| t a t t t t c g t t | a t t t t t a a g t   | a g g g a t g a g t   | c g g |
| g g g a g a t t g g | t a g t c g g c g t   | t g t t t a t t t t   | t t a |
| g t t t t t a a t g | t t t t t t t t t t   | t t g t t g t t t t   | g g g |
| a t t t t t t t t a | t t t a a t t t c g   | a g g a a g t t a t   | a g a |
| t t g g t c g t t g | t a a t t t a g g t   | t t t a t t g g a g   | a t a |
| t c g t g t a g g t | t a g t t t t g t t   | g t t a g a a a g t   | t t t |
| t t t c g t t t g t | t t t t t a t t t t   | a g t a t a t g t t   | a g g |
| g a t g g g g a t g | a a t g t t t g t t   | a a g a t a t t t g   | a t g |

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ggtttgtttt gtggggttaa ataggttttc ggtttaaata gagattattg agagtacgat 8820

|               |               |               |     |
|---------------|---------------|---------------|-----|
| gtgaagtgtt    | tatttgtgta    | aagtgttttta   | cgt |
| gtattttttt    | tttgtggttt    | tttcgattttt   | ttt |
| ggttgggggt    | tttgaatgtt    | ttttatgata    | tta |
| tgttagattt    | tttttagagta   | aagggtagcg    | gaa |
| tttgggttaa    | gtcgaatttgt   | ttttgtcgtg    | gat |
| atgatacggga   | attttgtttt    | tgttagatttg   | tag |
| gtatttaatat   | tttttgggttaa  | ggtaattgatt   | gaa |
| agtttttgtgg   | tttattttggg   | aggtttttttt   | ttt |
| ttttttttgtg   | agtttttataat  | ggtttggttttc  | gtg |
| tcgtaataatt   | taggggggttt   | ttgggtatcga   | gat |
| tttttgttttt   | agtttagggaga  | ggaggacggg    | ttg |
| gtaggggtttt   | aggagggtttt   | tgttagaggag   | gtt |
| agagagagaaa   | ggaaggggagg   | gtagtgttcgg   | ggc |
| tggagggtttt   | tttggggtgat   | tcgtttttagg   | agt |
| gtgagagggtt   | tttttttttttta | ggttttttgttg  | tgt |
| tttgcgagaaa   | tttgtattttg   | tttttttcggtg  | gtt |
| ttgtatggag    | atttttttttat  | tttgggggttt   | gag |
| tttgggggttt   | tagttttgttt   | ttaggcggtg    | ggt |
| tttgggggggtt  | tttcgggttgg   | agttatttttc   | ggg |
| ggtttttttttt  | ttttttttttttt | tatttttttgcg  | gtt |
| tttggggttga   | ggaaaattttta  | taattttttatt  | ttt |
| tttttttttttat | tacgtgggttt   | tttgtgggggt   | tgg |
| ggaaagggggt   | gtgttttcggg   | gaaagggtttt   | agt |
| aaatttcgttt   | gaatttttggg   | tttttttttttta | gtg |
| ttataattatt   | tttttttttttag | tgggggttggtt  | ttt |
| ttcgttttttt   | ttgtgattttg   | agttttgtgtg   | ttt |
| ttcgggttttgc  | gtttttttttttt | gtttttttgggtt | ttt |
| agattttattt   | ttagttttttttt | ttttttttaaat  | att |

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tagaggiggg ttttgggttc gaagttcggg tagaattttg gaggttagga tggtttgaat 10560

|                     |                     |                     |       |
|---------------------|---------------------|---------------------|-------|
| t t g g g a g g t c | g a g g t t g t a g | a g a g t t g t a a | t c g |
| g a g t t t t g g a | a g t t t g t t t t | a g a g t t a g t t | a a g |
| g c g t t a g t t t | t t t t a t t t a t | a a a a t g g g g g | t a a |
| t g a g a g a t t t | a a a t g a g g t g | g t g g a t t t g g | a a g |
| a g g t g t t t g a | t t t t c g g t t t | t t t t t t g t g a | a t g |
| t t g g g t t t t a | t t t t t t t t g a | c g t t g t t t t t | t t t |
| g g g t a g g t a g | a g a c g t t g t t | g t a g g t t a a g | g g c |
| a g t t t t a g t t | a g t t t g g a g a | t t t c g t g t t t | t t t |
| t t a g g t t t t t | c g t t t a g g g t | t a t t t a t a t t | a a g |
| g g c g g g g g a g | t t t t t g t t g a | g g t t t t t g t t | t g t |
| t t g t t t g g g t | t t g a a t t t a a | g g t t g g g g a t | t t a |
| g t t t a a t t t g | g t t t t t t t t a | g g g t g g a c g t | t a t |
| a g t t t t a c g g | a t t t g g t g g a | g t a t t t t a a g | a a g |
| t t t g t t t a t t | t g c g g t a g g t | t a g g g g t g g g | t t t |
| t t t t t t a g a t | g t g a g t t t t t | g g g a t t t t t g | a g t |
| a g t c g t a t t a | t g t t a c g a g g | g t g a a t g c g g | t t g |
| a t a a g a a g t a | g g a g t t c g a g | g a t a t a g t t a | a g g |
| a t g g t g g g g a | t c g g t a g g g t | t g g g g t a g t t | g a g |
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| t a g g g c g t t t | t t t t t t t t t c   | g t a t t c g t t t | t c g |
| g a g t a g t t a g | a t g t t a g g g t   | a g a a a g g g a t | t t t |
| a a a t t g a g g g | t t a g t g a t a a   | a g t t t c g a t t | a t a |
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| tttattttttt | aggatatattag | tgtttttaaat  | aaa       |                           |
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| g g g a g g t a g t                 | t g g g t t t a t t                     | t t t g a t t t g t                   | c g t |
| t t a a t t t c g t t t t t t g a a | a t g t t t t a t t a g g t t c g t g a | g g t t g t c g a a g g t t t t a a a | 7140  |
| t t a t t t a t t g                 | t g t a g c g t t t                     | a t t t t g g g g g                   | g a g |
| g t t t t t t t t t                 | t t g g g t t t t t                     | a g t t t t g a a t                   | t t a |
| a t a t t g t g g t                 | t a t a g a t a g g                     | a g t t t t a g t a                   | g a g |
| t c g t a t a t g a                 | t t t t g a t g t g                     | g g t g a t t t t g                   | a g c |
| t t a t t g a g t a                 | t a g a a a g t a c                     | g a a g t t t t t a                   | g g t |
| g t t t a g g g t t                 | c g t t t t t g g t                     | t t g t a g t a g t                   | g t t |
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| t t g a g t a g a g                 | a t a t t t a t a g                     | a g a g g g g t c g                   | g a a |
| t g c g t t a t a t                 | g t t t t t a a a t                     | t t a t t a t t t t                   | a t t |
| t a g g t a t g a t                 | a t t a t t t t t a                     | t t t t a t a g a t                   | g a g |
| g g t t t a g g g t                 | t t t t g a t t g a                     | t t t t a g g g t a                   | a g t |
| g t g t a g t g g c                 | g c g g t t a t a g                     | t t t t t t g t a g                   | t t t |
| t t t t t a g a g t                 | t t t a a t c g g g                     | t t t c g a a t t t                   | a g a |
| g t a a t a t t t a                 | a a a t a t t t g g                     | a g g a g a g a a g                   | t t a |
| t g t g t t t t a g                 | t a g a g a t t a g                     | a g g t a a a g a g                   | a a a |
| t g g t g g g a g a                 | t g g a t a t a t a                     | g a t t t a a g t t                   | a t a |
| a g g g a g g c g g                 | g g a a g a t a a t                     | t t t a t t g g g g                   | a a a |

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| tttttttattg | ttttaatatg  | tgttgggggtg  | agg             |
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| agtggttttt  | ttgttttttag | tgggatattgg  | gtt             |

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|                     |                     |                     |                     |
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| a g a t a t t a g g | t t t t a g g a t a | g t a g g g a g g a | g g g               |
| a g g g g a g g g g | a t g g a g g g t a | g g t a g t g t t g | g t t               |
| t a g g g t g g g a | g t c g g t t t a t | t t t t g t t t g g | g a a               |
| t a t t a g t g g g | a t c g g a g t a g | t t t a g c g g g t | a t t               |
| t t t g t a g g a t | a t t t t g t t g t | t g a g t g t a g t | a t t               |
| t t t t t t t t t t | a t a t a g g t t a | t a g a a a t t t t | t t g               |
| g a t t t t t t a t | t t t g t a g g g t | a t t a g g c g g t | g a g               |
| a g g g t a g g g a | t c g g t t g a g g | t t t t g g g t g g | g g g               |
| a g g g a t g g g g | a g t g t g g g g g | t t g g g g g t a a | g g g               |
| g g g a a t t a g g | a a t g a g t g g t | t t a g g g t t t g | g a t               |
| g g g t t g c g g g | g g g t t a t t t   | a t t g a c g g a   | g a g c g a g a a g |
| g g g t t g a g t t | a g g a a g t t a t | t t a t t t t g g t | t t t t g c g a t t |
| t t t a t t g a g g | t t t c g g t g a a | c g t g g a t a t t | t c g               |
| c g t g g g t a t t | a g g t a t t t t t | a t t a t t t g g g | c g g               |
| t t t t g g g g g t | t t t c g g a g a g | g t t t t t t g t t | t a t               |
| t a g g t a g a g a | g t t t a g g g a t | g a a g g g g g c g | t a g               |
| a t t t t c g g t g | g t t t t a g t t t | t a a g t t t t a g | a t g               |
| g g g g a t a g g a | a g a g g c g c g g | t g g g g t t g t t | a t t               |
| a g t a t a g t a a | g g g a a g a g a a | t c g a t t t t t t | g g g               |
| t t t t a t a c g t | g g g a a g a g a a | g t a g a g t a g g | g t g               |
| g t a g t a a g g a | t t g t a a t t a a | a g g t t t a g g a | g t a               |
| a a t t t a g a c g | t a t a t g t g t g | t t t t g t a t a t | t t t               |
| t t g g t t t a c g | g t t t t a c g g t | t t t t g t a t a t | t t t               |
| t g t c g g g c g t | t t a a t a a t a a | a t t t t t t t t t | t t t               |
| t t t t a t g a a a | t g t g t t a t a a | t g a t g g c g g t | g a t               |
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| t a g t t c g g t t | t g t t t g a g t t | t t t t a t t a t t | t t a               |
| a a t a g a g a t t | t t a a a g t t a g | t a t t a g t g a g | t t a               |
|                     | a a g a g t t t t t | t g t t t t g a a t | t t t               |
|                     |                     | t t g t t t a a a g | g g g               |

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| gttggttatttt          | tttttttttttt          | tttttttttttt          | ttt   |
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g t a g g g g a a a g g g a a g t g a a a g g a a a t a a a g a a

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&lt;210&gt; 18

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## 【図面の簡単な説明】

【図1】本発明にかかる造血器腫瘍細胞検出方法で用いられるSHP1遺伝子ゲノムDNAのセンス鎖の塩基配列を示す塩基配列図である。

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【図 2】図 1 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 3】図 1・図 2 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 4】図 1～図 3 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 5】図 1～図 4 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 6】図 1～図 5 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

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【図 7】図 1～図 6 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 8】図 1～図 7 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 9】図 1～図 8 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 10】図 1～図 9 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖の塩基配列の続きを示す配列図である。

【図 11】本発明にかかる造血器腫瘍細胞検出方法で用いられる S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列を示す塩基配列図である。

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【図 12】図 11 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 13】図 11・図 12 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 14】図 11～図 13 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 15】図 11～図 14 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 16】図 11～図 15 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

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【図 17】図 11～図 16 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 18】図 11～図 17 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 19】図 11～図 18 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 20】図 11～図 19 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖の塩基配列の続きを示す配列図である。

【図 21】本発明にかかる造血器腫瘍細胞検出方法で用いられる S H P 1 遺伝子の c D N A の塩基配列を示す塩基配列図である。

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【図 22】本発明にかかる造血器腫瘍細胞検出方法で用いられる S H P 1 蛋白質の概略構造を示す模式図である。

【図 23】図 22 に示す S H P 1 蛋白質のアミノ酸配列を示すアミノ酸配列図である。

【図 24】図 1 に示す S H P 1 遺伝子のゲノム D N A (センス鎖)において、C p G 島でメチル化される C G 配列の部位を示す塩基配列図である。

【図 25】本発明にかかる造血器腫瘍細胞検出方法で用いられる重亜硫酸処理にて、シトシンがウラシルに変換される過程を示す化学反応説明図である。

【図 26】本発明にかかる造血器腫瘍細胞検出方法で用いられる重亜硫酸処理により、シトシンがウラシルへ変換され、メチル化されたシトシンが変換されない状態を示す模式図である。

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【図 27】本発明にかかる造血器腫瘍細胞検出方法で用いられる S H P 1 遺伝子ゲノム D N A のセンス鎖に対して、重亜硫酸塩処理した後の塩基配列を示す塩基配列図である。

【図 28】図 27 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 29】図 27・28 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 30】図 27～図 29 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 31】図 27～図 30 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 32】図 27～図 31 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 33】図 27～図 32 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 34】図 27～図 33 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 35】図 27～図 34 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 36】図 27～図 35 に示す S H P 1 遺伝子のゲノム D N A におけるセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 37】本発明にかかる造血器腫瘍細胞検出方法で用いられる S H P 1 遺伝子ゲノム D N A のアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列を示す塩基配列図である。

【図 38】図 37 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 39】図 37・図 38 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 40】図 37～図 39 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 41】図 37～図 40 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 42】図 37～図 41 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 43】図 37～図 42 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 44】図 37～図 43 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 45】図 37～図 44 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 46】図 37～図 45 に示す S H P 1 遺伝子のゲノム D N A におけるアンチセンス鎖に対して、重亜硫酸塩処理した後の塩基配列の続きを示す配列図である。

【図 47】(a)～(d)は、それぞれ本発明で用いられるメチル化特異的 P C R のステップを示す模式図である。

【図 48】(a)・(b)は、本発明の実施の一例である実施例 1 において用いられる P C R 用プライマーを示す塩基配列図であり、(c)は、(a)・(b)で用いられる P C R 用プライマーが認識する S H P 1 遺伝子(ゲノム D N A・センス鎖)の塩基配列を示す塩基配列図である。

【図 49】(a)・(b)は、本発明の実施の一例である実施例 2 において用いられる P C R 用プライマーを示す塩基配列図であり、(c)は、(a)・(b)で用いられる P C R 用プライマーが認識する S H P 1 遺伝子(ゲノム D N A・センス鎖)の塩基配列を示す

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【図 50】(a)・(b)は、本発明の実施の一例である実施例 3 において用いられる R-T-PCR 用プライマーを示す塩基配列図である。

【図 5 2】 (a)・(b) は、本発明の実施の一例である実施例 5 において用いられるメチル化特異的 PCR 用プライマーを示す塩基配列図であり、(c) は、(a)・(b) で用いられるメチル化特異的 PCR 用プライマーが認識する SHP1 遺伝子 (ゲノム DNA・センス鎖) の塩基配列を示す塩基配列図である。

【図53】(a)は、蛍光 *in situ* ハイブリダイゼーション (FISH) を示す 10  
図であり、(b)は、ALL患者におけるSHP1遺伝子の異型接合性喪失の解析結果の  
一つの典型的なデータを示す図である。

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| oatutagtt  | gtgocogtg  | taguocaaat | gtoutctoo  | aaacocacg  | gacagagzo  | 60   |
| otgatogct  | oatitigat  | aaagatgag  | oagooagtg  | agagagagt  | tatgottag  | 120  |
| aatocoooc  | oatocagagt | tattagagaa | gagtgtout  | agagagagc  | ttagocctag | 180  |
| oatocotct  | gacagtatc  | ttaagttag  | togotttga  | aaatagtaa  | aaagocagaa | 240  |
| tootgagat  | gagocaggt  | caggaagat  | gaagagcgt  | tttgtogco  | oatagagc   | 300  |
| gocococct  | gagcttgct  | otagtacag  | cagtagocoo | tootgagat  | tgacootoo  | 360  |
| tttgtacgt  | gocagagag  | gaagagcgt  | gagttagtc  | aaatagtaa  | agatgootg  | 420  |
| aaatagcgt  | tgtgttagt  | otagtacag  | oatgacaaa  | oatoototo  | otkocotgt  | 480  |
| taoototgoc | ttttagagoo | oatococot  | gaagotctc  | toottagct  | oatgagtag  | 540  |
| oatagocgt  | oocagagoc  | taacotacg  | tgaagtctc  | oatootocoo | oagagaggt  | 600  |
| tgagagcgt  | gagttagtc  | otcagocoo  | tgtatgttg  | oatgacaaa  | gaatagact  | 660  |
| oatitotag  | gaatagagc  | tattagagt  | tttotoagc  | oatcttagt  | tcogotagc  | 720  |
| gaagatttg  | oatoottagt | tgtotctgt  | cagagocgt  | taattotgt  | tgtatgooo  | 780  |
| taacagatc  | oatgagcgt  | tgtatggtg  | agtcocagt  | gaatogctc  | gtgtotctc  | 840  |
| oatootagc  | gaagagagc  | oatgagcag  | cagagagoc  | tgtatgctc  | oagatttg   | 900  |
| gctgagagc  | gtatgagc   | tattagagt  | otcagagoc  | oatgococaa | oatgtctct  | 960  |
| tootgagag  | tattatgac  | gaatttgac  | oatgacagc  | ATTGAGTGC  | CAGCAGTGA  | 1020 |
| CAGCAGTGA  | GTGCTGATT  | ATTGAGCAGT | TGCTTGTCAC | TGCTGTGCG  | CAGCAGTGA  | 1080 |
| CAGCTGTGCG | ATTGCTGACA | GCCTGCCTGC | TGCGGCGCTG | CAGCTGTGCT | TGCGCTGCG  | 1140 |
| TAGCAGTGA  | TGCTGTGCG  | TGCTGAGT   | oatgagcaca | oatgtctct  | agtcotagc  | 1200 |
| tattitgag  | gagtagagc  | gattitgag  | tgtotctgt  | gaatgtatg  | oatgagtag  | 1260 |
| oatitotag  | oatgocotgt | oatgtttag  | gctatagac  | oatootout  | oatgagagt  | 1320 |
| gtatgagoc  | oatgocagt  | oatgtotao  | tgacootat  | ttaactatg  | oatgagcga  | 1380 |
| agtgatgac  | tgttotoat  | atgtatgct  | taotacagc  | otatgagct  | otatgtatg  | 1440 |
| ototgocgt  | gtacootgac | oatgtgttg  | otcagacaa  | otcagacaa  | gaatgagc   | 1500 |
| gacocagag  | gaatgocac  | oatgtacag  | oatootatg  | gctatagac  | aaatagagt  | 1560 |
| tttgtatgt  | otitttttgt | tootcttag  | taactotac  | otgatagct  | caatagac   | 1620 |
| gtatgattg  | agatbotat  | atgacagag  | tgtatttga  | tgtatgtag  | aaatagagt  | 1680 |
| gaacagagc  | gaatagtgt  | otgtotacg  | agagctaac  | ttocagtag  | agattacag  | 1740 |
| cagacagct  | aaacocacg  | atgttagct  | tgaagtatt  | cagagattt  | oatgagaaa  | 1800 |
| tgagagcgt  | tgatgagag  | tattgttca  | oatcaggt   | oatgagtag  | tgagagagc  | 1860 |
| agtagagag  | atgttgagc  | oatgagagt  | tgagacagc  | oatgacata  | tattagagc  | 1920 |
| otgtototac | aaatagaaa  | aaatagat   | agattgttg  | cagagcgt   | gtattacag  | 1980 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| otatagagag | gotagagag  | gagagagag  | ttagagagag | agagagagag | ttagagagag | 2040 |
| gatagatag  | oagagagag  | oagagagag  | oagagagag  | agagagagag | toagagagag | 2100 |
| aaagagagag | aaagagagag | oagagagag  | ttagagagag | ttagagagag | ttagagagag | 2160 |
| atagatagat | agagagagag | ttagagagag | oagagagag  | ttagagagag | atagagagag | 2220 |
| agatagagag | aaagagagag | ttagagagag | oagagagag  | oagagagag  | atagagagag | 2280 |
| gagagagag  | ttagagagag | aaagagagag | ttagagagag | aaagagagag | gagagagag  | 2340 |
| oagagagag  | agagagagag | ttagagagag | aaagagagag | agagagagag | oagagagag  | 2400 |
| agagagagag | ttagagagag | aaagagagag | agagagagag | agagagagag | atagagagag | 2460 |
| oagagagag  | agagagagag | gagagagag  | ttagagagag | gagagagag  | oagagagag  | 2520 |
| aaagagagag | agagagagag | atagagagag | aaagagagag | atagagagag | oagagagag  | 2580 |
| ttagagagag | agagagagag | gagagagag  | ttagagagag | atagagagag | aaagagagag | 2640 |
| gagagagag  | oagagagag  | gagagagag  | oagagagag  | oagagagag  | atagagagag | 2700 |
| atagagagag | ttagagagag | ttagagagag | oagagagag  | oagagagag  | ttagagagag | 2760 |
| agagagagag | atagagagag | oagagagag  | oagagagag  | atagagagag | ttagagagag | 2820 |
| gagagagag  | oagagagag  | aaagagagag | oagagagag  | aaagagagag | aaagagagag | 2880 |
| gagagagag  | aaagagagag | gagagagag  | aaagagagag | gagagagag  | aaagagagag | 2940 |
| oagagagag  | gagagagag  | gagagagag  | aaagagagag | aaagagagag | aaagagagag | 3000 |
| atagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3060 |
| gagagagag  | aaagagagag | oagagagag  | aaagagagag | aaagagagag | aaagagagag | 3120 |
| ttagagagag | aaagagagag | gagagagag  | aaagagagag | aaagagagag | aaagagagag | 3180 |
| atagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3240 |
| oagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3300 |
| aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3360 |
| oagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3420 |
| gagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3480 |
| atagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3540 |
| oagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3600 |
| aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3660 |
| gagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3720 |
| atagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3780 |
| oagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3840 |
| aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3900 |
| gagagagag  | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 3960 |
| atagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | aaagagagag | 4020 |

|            |             |            |            |            |             |      |
|------------|-------------|------------|------------|------------|-------------|------|
| tsagtottag | tttotgtitto | tsaasttgg  | tsaasttaao | otototagta | szsttggoot  | 4020 |
| gagatattat | agttatsttt  | asaagttgt  | agototgtta | oottgoooot | tacagotott  | 4060 |
| tcaosttagt | attttgggaa  | tttttttag  | otototgtt  | agggagatt  | totagatotto | 4140 |
| agaagattto | ogagototto  | tacagatttt | tttgtotgtt | timgototto | agagotoota  | 4200 |
| totottttta | tsaootttga  | totgtttgt  | ttgtattttt | attttttttt | tttttttagaa | 4260 |
| agattttiao | totttgttoo  | aggtotagt  | oggttggoot | ggtotagott | caotgaoao   | 4320 |
| toaoottott | gagaotgtgt  | gutttaoogo | tagttagag  | aggttttiao | tttgttgoao  | 4380 |
| agotgtotot | gaoototoga  | ootagattgt | toototoooo | togoototot | asaagtgtto  | 4440 |
| gatttagagt | gtaagttagt  | gogooagott | attttttttt | tttttttaga | aggtttotao  | 4500 |
| totgtttoo  | agggagatt   | aaotgttga  | aaootgttt  | caatgttgo  | tggaootao   | 4560 |
| oaggtttag  | ogattotooo  | ttotagott  | ootaagtao  | ttgggttao  | aggtttgtot  | 4620 |
| ooaoootot  | gottaatttt  | gtatttttt  | ttggagoga  | gttttotoot | gttttoagtt  | 4680 |
| otgtototao | attttgttto  | agaattaoao | agoototao  | ooaoootag  | ttgttagatt  | 4740 |
| oaaggttgo  | gaoootgtto  | otgttotoot | ttototattt | tattttotgt | ototaoaga   | 4800 |
| aattttttt  | tttttgtatt  | tatagattgt | ttaotgttt  | attttttgt  | ootootag    | 4860 |
| tttgttogo  | ootaagaana  | atggggana  | ttattotoao | atooaggtto | tgttttagg   | 4920 |
| otaaataga  | totgtttatt  | gagattgtt  | ttttagooo  | aaoototogo | agatttaggt  | 4980 |
| agatttttt  | ttttttttot  | gootaagatt | agaaagatt  | gtattttgt  | tttgtattgt  | 5040 |
| gtototgtt  | tttttagao   | toagagatt  | totototott | toattgttto | agaaattagt  | 5100 |
| gaggttagt  | tttttttgt   | gttgootttt | attttttott | ooaatgtgt  | tttttagoo   | 5160 |
| ttggaggtt  | gttttttago  | oaggttttag | ttgggtttg  | gttttagtgo | ttgoottag   | 5220 |
| tttttttgt  | ttgttagatt  | ttgttttoat | gttttagatt | gtttagoat  | ootgtttgt   | 5280 |
| tttttagttg | aaagttaga   | ttaggtttgt | gttttagtt  | ttgttttago | oottttgo    | 5340 |
| oatttagaaa | gaggtttgt   | tttttagao  | aaagaggtt  | taattttgt  | oaggtttgt   | 5400 |
| gatttaggt  | gagaggttt   | tttttttag  | oottttago  | toagggatt  | ogoottttag  | 5460 |
| tttttagoat | ogaggttag   | ogaggttoo  | ogaggttto  | ogootttgo  | gogagtttt   | 5520 |
| oaggtttgt  | totgtttot   | ttaggtttgt | atttttagt  | ootgtttgt  | ootgtttgt   | 5580 |
| tttttaggt  | ootaggttag  | aggttagtgo | aaaggttago | agttagtaaa | ootooottag  | 5640 |
| agtttotoo  | gagagaggt   | gagagagao  | aaagagao   | aaatgttaga | aaagagaggt  | 5700 |
| agaaaggtt  | gaaagagott  | tttttttaga | aaagattgt  | gtttttgtt  | totaaaggt   | 5760 |
| taattttgt  | tttttagaa   | aaatttago  | ttttttgt   | aaoottttt  | oottttttag  | 5820 |
| gttagagaa  | aaatgttag   | tatttttaa  | tttttagaa  | aaaoottgtt | toatttttt   | 5880 |
| ttttttttt  | taagattgt   | ottttgttt  | tttaggtgt  | gttttaggt  | gtttttgtt   | 5940 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| agotogotgo | asototogot | tootggotgo | asgtatgot  | otgototgaa | ootooogagt | 8000 |
| agotggotgt | acagotogot | oocooatgo  | ogootatgt  | ttttttgt   | ttttatgat  | 8080 |
| agagtttgo  | oootgttgg  | ocagatgtt  | otugtotoo  | tootogotg  | otocogotgo | 8120 |
| otggotogot | oasagtgtg  | gattatogag | otatogooo  | tgogooogot | ooatootag  | 8180 |
| tttatataca | oatgotogaa | cagatogot  | atagatocaa | agagottoo  | otatootoo  | 8240 |
| asagtittoc | agaaatgtt  | oocagttag  | atatatttt  | ttatataggt | asgototgo  | 8300 |
| oototatat  | oottataaa  | gattgtatg  | gaaoogagt  | totoocooat | ooitgotot  | 8360 |
| cagocococa | gtittotoot | otaggagot  | oocaaatgt  | atttttota  | ttatotoot  | 8420 |
| gttgatogot | ttttotogot | tttgtttgt  | ogstgttgt  | gttttttgt  | ggattatag  | 8480 |
| gttagagat  | tatatatoot | tattgtttg  | goototbag  | atocaaocoo | oottagaaa  | 8540 |
| toagocotg  | tgagagoot  | gtocooocoo | oocooagga  | tagocoooot | asggagatg  | 8600 |
| otaggagot  | ggagagotg  | agagacocoo | atagagag   | oocootgtg  | oogagotg   | 8660 |
| toagatnot  | tttgtatoot | oototocoo  | asgtogag   | acagotgtg  | tttgttgtt  | 8720 |
| agagagotg  | acootttot  | otggagocoo | ttggagagt  | otaaatoot  | agtototgo  | 8780 |
| toootgtgo  | ooottttgt  | oocagaggo  | tottatoot  | tttttttgt  | agoottagg  | 8840 |
| gotgtgtat  | agagtttaa  | agcaulgot  | ttggaoogg  | asgtotggg  | tttgatoot  | 8900 |
| agoototag  | otatatoot  | gattagocoo | gaaatnot   | taaatootg  | agagatnot  | 8960 |
| tttoattgt  | gttgtatgt  | asagatgao  | oottttgtt  | agagotgtg  | tgagaoocoo | 9020 |
| atagacagot | gttgtgaao  | goottatat  | cagagagoo  | gaaatagag  | agagatnot  | 9080 |
| ogwtgocagt | ttgtocogoo | atatgttga  | ttagotgg   | oocagaggg  | otagataaa  | 9140 |
| gagotgtg   | ggagagagoo | agagootgt  | gagootgtg  | ggttgotag  | otagagtat  | 9200 |
| ttatogtgo  | tgagatgtg  | agagococoo | tttgtotaa  | otatagtgt  | ttgtatoot  | 9260 |
| gtagatogot | ootatogoo  | goottttgt  | gagagatgt  | agagagag   | oocogtoco  | 9320 |
| oocooocotg | oocoototgt | ototttotgt | tootottgt  | totatataa  | ogagatagot | 9380 |
| asgtagotg  | oocooagag  | toagocogoo | otototoot  | oocogotgt  | ogagotgooo | 9440 |
| cagagooAGT | GAATGGAGG  | CCGAAAGTG  | GGAGCAGGG  | GAGTGTGAG  | GCGGCGCGG  | 9500 |
| AGTGGAGAT  | GCATGTGAG  | ATTATAGTGT | AGAGTTTGT  | GTGTCCGAG  | ATGATGTGAG | 9560 |
| CTGTGATGT  | CTGTGGCGG  | GTTCTGTGTG | GAAGACCGCG | AGATGTGTGA | AGAGTGTG   | 9620 |
| CCGACCGCG  | ATGAGCGAG  | GGGAGAGAG  | GGTGGTGG   | oagagagag  | ootototot  | 9680 |
| oototogotg | ggagagooag | TGTTTTCAG  | GAAGACTGAG | TGGATGTGAT | GCAGAGACCG | 9740 |
| TGTTGAGAG  | CGAGAGTGT  | CAGGTTAGT  | TGTTGAGTG  | CGGAGATGCG | AGAGACGAG  | 9800 |
| GTGACTTGT  | GTCTGTGAG  | AGagagag   | gocococoo  | aoocogogoo | tttttgaoa  | 9860 |
| totttttgt  | ootatogotg | otatocoot  | cattotogt  | tootogotg  | asgtatnot  | 9920 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| oocogtotgt | toocitgoc  | ocaaococaa | caotococat | cootgtotgt | gocoooccat | 7980 |
| gocootgtgt | gocooococ  | agacootag  | ocagttoocg | cootocotoc | toatootgt  | 8040 |
| caocogtggt | ocococococ | tgtgtocgt  | capgg7gggg | gATCAAGTg  | OCGATATTGg | 8100 |
| GATCAGCAG  | TGATGGAGT  | TOTATGAGT  | GATAGAGgg  | GAGAGATTGg | CAAGCTGACg | 8160 |
| AGAGCOTGGT | GAGTACTACA | TCAACAGAg  | gggTGTGTGg | CAGAGACAg  | ACAGCAGCAT | 8220 |
| GATCAGAGT  | AAATAGCgg  | GTAGCTGTG  | CGATCCAGT  | AGTAGAGgt  | gagggtocag | 8280 |
| ocacocogoc | gttccocag  | agggatgag  | aggtocococ | ocagagagoc | caggagggoc | 8340 |
| gagagocgg  | gacagggag  | tgacotacot | coacotococ | tootocotgt | acacogagg  | 8400 |
| gufotacgt  | tootocotoc | otgtgttoot | ggagootgt  | gtotacagp  | ctaacotoc  | 8460 |
| acootttoc  | otacacococ | agggagagoc | agagagagoc | ocotocotac | toaggagoc  | 8520 |
| ctggocgtg  | caacocagt  | nocootggg  | anagaggag  | catogtggt  | ggacogagt  | 8580 |
| tgtagagag  | ocagotgtgt | gttagagag  | tottottoot | otagtagoc  | gootgooto  | 8640 |
| otocotgtgt | cootacococ | agacagtgt  | agacagtg   | gagtagac   | ctagtgtga  | 8700 |
| gttagtggt  | agtgttgag  | agacagotg  | atgtgtgtg  | ccagocpoc  | ggagagoc   | 8760 |
| gtgtgttoot | gttaggtoc  | atagttotg  | ggococacoc | gagatocgt  | ggagocagt  | 8820 |
| gttagtggt  | caactgtg   | agtgtotac  | aggtatocg  | ggocagagt  | atagttoag  | 8880 |
| goottttoot | otagtgtoc  | ococogotoc | tootgttgt  | toocacagag | atggtaggt  | 8940 |
| gttagggag  | totagtgtg  | coctatgac  | coctagtto  | tttagagoc  | goototocg  | 9000 |
| tgacagatoc | occttaggt  | agggagagc  | gataagagt  | aggggtttt  | caagtgcac  | 9060 |
| goottgocaa | ggocotgtoc | coittgagt  | gacotococ  | tgatgttag  | gatttgtag  | 9120 |
| atagtagoc  | coittgtoc  | tgacogtgt  | agacototg  | taacgttag  | otococagt  | 9180 |
| gacatagat  | tttttgtaa  | ggocagagt  | gaacataga  | gattgttgt  | gtacogtag  | 9240 |
| agoottgag  | ccocootgtg | aggottoct  | tgatggag   | goottoctg  | agoottooc  | 9300 |
| totototgt  | agootacat  | gggtgtotoc | gtgtotgoc  | coitgooto  | ototococg  | 9360 |
| ocogacagat | ccaggggtgt | tgagacag   | gacoottag  | agutagtgt  | ototottott | 9420 |
| otogootoc  | agagagggg  | ggagggag   | ctagagagc  | otaggggtg  | gagagggag  | 9480 |
| ggagggococ | agagagoc   | tgagagag   | gutaagagt  | taactagag  | agagagag   | 9540 |
| agagagag   | ggagagag   | gactagocg  | ggagaggt   | taagagagc  | gagagocag  | 9600 |
| tgagggoot  | tttagtgag  | coitgocag  | agacagtgt  | gocootgag  | otggagagt  | 9660 |
| gttagaggt  | otittotocg | gattgtotg  | tgatottotg | otattgtgt  | goottootoc | 9720 |
| totogagag  | tttgtotag  | tototoggt  | gootogotgt | tottgtgtg  | agootagat  | 9780 |
| tgatagag   | atootacot  | ocotagoot  | gagagggag  | gagagococ  | otocootgta | 9840 |
| cootagttag | taacotctac | ccacogagt  | grottagta  | gocacagt   | attagagag  | 9900 |

|            |             |             |            |            |             |       |
|------------|-------------|-------------|------------|------------|-------------|-------|
| ttotzggzgt | totoztogtzg | agtozootoo  | ggzozggzgt | gatzgtzgtt | ggzazagzgt  | 9980  |
| gztotogzgt | ttotzotooz  | ootootogtz  | gtgzamatz  | ttgzoogzoo | ttotoottgt  | 10020 |
| ootzgtzoo  | ggzootooz   | oazootoet   | ttogoozoo  | gtzgtttgtz | gattgtttgt  | 10080 |
| ttttttotz  | oazgtgttt   | ootzgtggz   | tgzgtttgt  | ggzgtzooz  | tttoototgt  | 10140 |
| ggzazggzgt | gtzgtotgz   | gaaazggzgt  | gztgtotgt  | tttgozgtz  | oazgootto   | 10200 |
| azotzgttt  | gaaazogzgt  | ootoototz   | gtgzootatz | oazggzooz  | oazgambzoo  | 10260 |
| otazootzgt | otttotooz   | tgzgtttgt   | tttgoozgt  | ootzgtgz   | ggzoozooz   | 10320 |
| ttotzootgt | tttgozgt    | gztgtttgt   | toozotooz  | oazootoot  | gtzgtttgt   | 10380 |
| otzgtzotgt | gttttoottt  | tootzgtz    | toztgtzgt  | oazootooz  | toottzggz   | 10440 |
| agtttoozoo | ttzgttoott  | tootzoozatz | atttggatz  | ttgzoogzgt | ttotgtott   | 10500 |
| oazgtzgtz  | ootzgtzgt   | gaaazoozgt  | tgazootzgt | gatzgtzgtz | tgzgttgzaz  | 10560 |
| otzgtzgtz  | ggzgtzgtz   | agazgtzgt   | oogzoozot  | gaaotzgtz  | otzggzazaz  | 10620 |
| gtzgtzgtz  | agzgttozgt  | agzgtzgt    | agggzoozgt | oozggzgtz  | tgazootzgt  | 10680 |
| gztgtzgtt  | ootzototzt  | azatzgtzgt  | ttatztoatz | ootzgttoz  | gzoztgtzgt  | 10740 |
| tgzgtzgtot | azatzgtzgt  | gtzgtzgtz   | agzotzgtz  | ootzgtzgt  | ggzoozazgt  | 10800 |
| agztgtzgt  | tttggzooz   | ootzgtzgt   | gtzgtzgtz  | toazgoott  | ootzgtzgtz  | 10860 |
| otzgtzgtz  | ootzootzgt  | ootzgttoz   | toztgtzgt  | AGAZTGBCA  | AGATTOTGAG  | 10920 |
| GGGACGAGAG | AGAGCTGTCT  | GAAGAGCGAG  | GGAGGCGCGT | CGAGTTGT   | TTGTGTGTAG  | 10980 |
| AGCTTCAGCG | AGGCTGTGAG  | TTTCTGTCT   | TTGTGTCTCA | GTAGAGAGCG | GAAGGCTGAG  | 11040 |
| CGAGGTCTCG | GGCTGAGGTT  | GAGCCAGAT   | AGAGTCATGT | GGAGAGTGT  | ggzoozazgt  | 11100 |
| ggzggzggz  | gztgtotgt   | gztgtotgt   | ttzgtzooz  | gtzgtzgtz  | oazggzgtz   | 11160 |
| otzgtzgtz  | gtzgtzgtz   | ggzggzggz   | oazggzggz  | agzootazgt | ootzgtzgtz  | 11220 |
| gzoattgtz  | gtzootooz   | ggzgtzggz   | TAGAGAGCG  | GTGATTTGGA | GAAGCTTGAG  | 11280 |
| AGCTTCAGCG | AGCTGTGTGA  | GAATTTCAG   | AGAGAGGGGA | TGAGAGAGCG | GTACAGGCGC  | 11340 |
| TTTTGTACG  | TGCGCGAGT   | ggzgtzgtz   | oazggzgtz  | toozootzgt | ootzgtzgtz  | 11400 |
| toozootzgt | ggzgtzgtz   | gatzgtotgt  | agztgtzgt  | ttotzootgt | toozoozooz  | 11460 |
| agzgtzgtz  | TTGAGAGAG   | GTGAATTGGA  | GTGATTCAT  | GAAGAGAGT  | TGTAAGATTA  | 11520 |
| AGAGAGAGT  | GTGATTCGAG  | GATGAGAGCA  | AGAGCTGGCT | CTGAGAGAGT | TTTGTAGtz   | 11580 |
| gtzgtzggz  | oazggzggz   | tgzggzggz   | ggzgtzgtz  | oazggzggz  | ggzoozooz   | 11640 |
| oazggzoot  | ootzgtzgt   | oazootzgt   | ootzgtzooz | oazootzgt  | agzootoozgt | 11700 |
| atzgtzgtz  | tttotooz    | atzgtotz    | tttotoozoo | oazootzgt  | AGTTTTCAGAG | 11760 |
| AGAGAGAGT  | GAAGAGAT    | CGAGAGCGT   | TGGAGGGGCA | GGGAGAGAG  | AGAGAGGGCA  | 11820 |
| AGAGAGGGTA | GAAGAGATT   | CGAGCGGzgt  | oazootzgt  | otzootzgt  | oazootzgt   | 11880 |

【图 7】

|             |            |            |            |            |            |       |
|-------------|------------|------------|------------|------------|------------|-------|
| acagacacacg | ccacagcgta | ctacacacac | ctacacagtc | tccacacac  | acacacagac | 11040 |
| agacacacac  | ccacacacac | ccacacagac | tccacacac  | ccacacacac | tccacacac  | 12000 |
| ccacacagtc  | ccacacacac | ccacacagac | agtcacacac | tccacacac  | ctacacagta | 12060 |
| ctacacacac  | tccacacac  | acacacacac | ccacacac   | ctacacacac | tccacacac  | 12120 |
| ccacacac    | tccacacac  | acacacac   | ctacacac   | agtcacac   | ccacacac   | 12180 |
| agtcacac    | agtcacac   | acacacac   | ctacacac   | ccacacac   | agtcacac   | 12240 |
| acacacac    | tccacacac  | acacacac   | acacacac   | agtcacac   | ctacacac   | 12300 |
| AACTAAGTA   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | 12360 |
| AGTCACAG    | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | 12420 |
| ctacacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 12480 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 12540 |
| AGTCACAG    | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | 12600 |
| AGTCACAG    | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | 12660 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 12720 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 12780 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 12840 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 12900 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 12960 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13020 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13080 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 13140 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 13200 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13260 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 13320 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13380 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 13440 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13500 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13560 |
| ctacacac    | ctacacac   | ctacacac   | ctacacac   | ctacacac   | ctacacac   | 13620 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13680 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13740 |
| agtcacac    | agtcacac   | agtcacac   | agtcacac   | agtcacac   | agtcacac   | 13800 |
| CTCTCTGAC   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | AGTCACAG   | 13860 |

【图 8】

|             |            |            |            |            |            |       |
|-------------|------------|------------|------------|------------|------------|-------|
| GTCCGCGGCTG | GACAAATgta | gtgacgcccc | ggaactggcc | cccttcgggg | gtctctccct | 13829 |
| ggactgttt   | tcctctatgt | tcagctgttg | tgagactagt | gaggttatgt | gagagagagg | 13890 |
| gagacacgtg  | ccactgtatg | tcacttgatg | AGGAGGTGAT | TCGGGAGATG | TGGCATATGC | 14040 |
| AGTACGATG   | CTACGCGCAG | CATGAGGTGC | CGAGTABGCG | TGGGGGTGTG | CTGACATGTG | 14100 |
| TGACACAGAT  | CACCCAGCAG | CGAGAAATGT | TCGCTADACG | AGGACCCATG | ATGCTGCATG | 14160 |
| GCAGStgtag  | gtgatantcc | tgctagtgt  | agtgcacagt | gagagataga | taotgtatg  | 14220 |
| tgcactgag   | tgattatag  | actatanaag | tcagctatg  | actattgtg  | cctgtctctg | 14280 |
| cccccgtgtt  | ctctatgctc | ccctatcgtg | tcagtaaccc | tgagtgatga | tgctgtgaga | 14340 |
| ccagcagccc  | tttgagctgt | tgctgtgttg | ttctctcttg | agagctctgt | ccgagatctg | 14400 |
| ctctctctgg  | aacctctgca | actatagagg | tttggcagtg | gagacatttg | ctgtgcagtg | 14460 |
| ccagatagt   | cagggcagag | ccctcagcag | ccccacgtg  | gagctgttgc | cctgtgctgt | 14520 |
| ccgtttctct  | agagctgctt | ttctctgttg | ttctctcttg | agagactcgt | atggagctgt | 14580 |
| tgctccactc  | tcctctctct | ttctctctgt | tcctcagag  | tcagctgtgt | ctctctgctt | 14640 |
| ctgctctctg  | tcctagctgt | ccagagctgt | gccccctgt  | gccccccctg | actatgtttg | 14700 |
| ctagtgtgt   | gctttctgtg | ccctctccct | actctctctg | tcctatctac | ccagatgttg | 14760 |
| tgctcaggag  | acotctgttg | agctcagag  | tgagctgtg  | ccagagagag | cagggtttcc | 14820 |
| ccagctgtag  | ccactgtagt | gctctctgt  | gagctatga  | gcagctgtg  | gtgcagccag | 14880 |
| ctctctctct  | ccctctctgt | ctgtcttgag | actatcttta | actttcttta | ccctctgctt | 14940 |
| ctctctctgt  | aacctctaga | tcctctctgt | ctgtctgaga | ctctctgtga | ctctctctgt | 15000 |
| tgctctctga  | tcctctctga | ctctctctct | ctctctctct | tcctctctta | ctctctctca | 15060 |
| tcctctctag  | actatgttag | ctgtctctct | ccctctctgc | gctctctccc | actctctctg | 15120 |
| tgctctctag  | ccctctctgt | ccctctctgt | tcctctctct | ccccccctgt | ctgtttctgt | 15180 |
| ttgcctctag  | ccactctctg | gactctctgt | gctctctctg | tgagctcttg | agagctcttg | 15240 |
| ctctctctgt  | tcctctctca | ctctctctga | ccctctctcc | gctctctctc | ctgtctctcc | 15300 |
| gctccactca  | ccctctctca | ctctctctga | gccccctgtg | ctttactgt  | agctctctga | 15360 |
| agagctctg   | gtctctctga | ctttctctg  | actctctgt  | tgactctgt  | ccagctctga | 15420 |
| tcagctctgt  | agctctctgt | gctctctgt  | ccctctctgt | ggagctcttg | tgagctctgt | 15480 |
| ttgcctctag  | ctgtctctag | agagactct  | ctctctctgt | tcctctctct | agctctctga | 15540 |
| ctctctgccc  | ccctctctgt | ctgtctctgt | gctctctctt | ctctctctgt | agagctctga | 15600 |
| gagagctctg  | agtctctgca | tcctctctga | ccctctctga | ctctctctga | gagagctctg | 15660 |
| tcctctctag  | ccctctctga | ccctctctga | ccctctctga | gattctctga | tcctctctgt | 15720 |
| tcctctctg   | agctctctcc | tcctctctga | tcctctctgt | gcctctctga | tgagagctga | 15780 |
| aacctctgt   | ccactctctg | ccctctctgt | ccccccctgt | ccccccctgt | agagctctga | 15840 |

【图 9】

|            |            |            |             |             |             |       |
|------------|------------|------------|-------------|-------------|-------------|-------|
| atcagcagag | tgcccaaaag | tccatctatc | cttgcccggt  | gacccctgga  | acattccact  | 15900 |
| ccatccctgc | acgtccagcg | ttgcctctgt | gtgcctgtgg  | ctctggctgct | acgtctggac  | 15960 |
| cccccctccc | cccccctccc | tgtccctggc | tctgtctgac  | cccccccttt  | tcoccccgcc  | 16020 |
| ccgcctctgc | ccgcacacgc | ccatcattgt | ctgcacacgt  | ctgcctgaga  | acatctccac  | 16080 |
| caagctgac  | cccccctccc | tgctttggcc | ctctggctgg  | acgcctccct  | ccgtctggcc  | 16140 |
| ctctgcctgg | cccttgagtt | tgactgcccc | cccccacggc  | ctgcagcttg  | acatttcacat | 16200 |
| ccagaaacac | ctctctctgc | tgccgcggcc | tgccgcggcc  | atgtgttcaga | ccctgcggga  | 16260 |
| ctacacattt | attctagctg | ccatccggcc | gtttatttba  | acattctaga  | aaagctggca  | 16320 |
| gtctctcgac | ctctgtctgc | acagccctgt | cccccccttt  | cccccccttt  | tcacgtctgt  | 16380 |
| gtcccccctg | ccctctctgg | gaccccaccc | ttcccccctg  | ccccctgccc  | aaagtctcag  | 16440 |
| aaagcccgag | actgcgacta | ccggaacatc | acgtattccc  | cagccactga  | caattcccat  | 16500 |
| cccaaacctt | ccgcacacgc | gtccacatga | gtgcctctgc  | ctgcctccct  | ccggtcccca  | 16560 |
| cccccttgta | ctctccagcg | cgtctccctg | ttctctggag  | cccccaagtt  | tcagactgtg  | 16620 |
| ggacccctgc | ctctctctgg | gtcttgctgt | tcaccccctt  | tgctttccct  | ctttctctgc  | 16680 |
| gtgcctccct | ctcttgagct | ctctctgtgt | ccacccagcg  | ccctctgtct  | gcagcttggc  | 16740 |
| ctctctctct | tgagctgtg  | gcgtctgtgt | cagggcccggt | ccgtgtctct  | ccgtctggag  | 16800 |
| ggctctgcca | cccccccttt | ccactgtctg | gtccccctgt  | ctgtgtctct  | ccgtccgccc  | 16860 |
| acactgtgtt | acttgcctcc | ctctcccgcc | ctgcacacac  | aaagctggca  | tgattctgaa  | 16920 |
| ctctccactg | aaagacacga | ccggaagaga | acttgcacac  | cagcgtctcg  | cacagacaga  | 16980 |
| gaagacacaa | gatttctgca | agagaaagtt | acccgtgtgt  | tccttcaggt  | cccatctgta  | 17040 |
| acactctgtg | ctctctgtga | ctccctggc  | tgactctgtg  | ccctgtctct  | actgtccctt  | 17100 |
| ctctctgtgt | ccagcttgcc | cagctcattt | ctgtctctgt  | ccctgtccct  | acacactctt  | 17160 |
| gttccactgt | ccagcttgcc | ctccctctct | ccctccctcc  | ccctctcttt  | tgagcgtct   | 17220 |
| acccctctgc | ctcttcagaa | atttccacat | ccgcacacgc  | acacactgaa  | ctcttcagaa  | 17280 |
| cccatctgtt | ttgttatatt | aatgcctgca | tgcccacacg  | ctgtccctga  | ccctcttatat | 17340 |
| acagacacga | ccgcacacga | agccgacacg | ctttctctct  | tgattataaa  | ccctgtccag  | 17400 |
| catctctgtg | ccctctctgg | cccttctgtt | ccccccctgt  | tgcttcagca  | gagagccgct  | 17460 |
| acagctctgt | actctctgtg | ctctctctgt | ctctctctgt  | tcagctctgt  | gcctctctgt  | 17520 |
| agacccctgt | ggctctggcc | tcctctctgt | cccttgaggg  | gtgcctctgt  | gtgcagctct  | 17580 |
| cccatctctt | ttcttatatt | ggagctctgt | ctaccacacg  | tgctttctgt  | ggctccagcc  | 17640 |
| atgcctctgt | ccgtcttgac | ggacacagtt | actctccctc  | ctctccctgt  | ctgcacagcc  | 17700 |
| ccctaacagc | tcacccctct | actgtctgtt | agacactctt  | gtctctctct  | tttctctgtt  | 17760 |
| ccccccctgt | ctcttcagaa | ccactctctt | actctctctt  | tcacccacag  | tcacacacct  | 17820 |

【図 10】

|            |            |            |            |            |            |       |
|------------|------------|------------|------------|------------|------------|-------|
| atgagacagt | gaggatgaa  | ttagaacocg | gvgtctgt   | ccgcagagcg | aaacactcct | 17889 |
| aaatgagctt | tgcagctgt  | gcacacatcg | ttgagcgccg | gctacacacg | aatctgctgt | 17940 |
| ctgttagcga | ttgtacacta | gagatgctgt | tacagcgctg | gagagcagca | ctgagcgacg | 18000 |
| ccacgctgtg | tcctctctgt | ggcgagacag | gacagctgtg | tacagcgacg | ccgcagcgag | 18060 |
| cagactctac | acatcatatg | cgctgcgtgt | ctctatctct | attgagagag | gtttgacaga | 18120 |
| ctgctgtgac | tgaagcagtc | ttgttttttt | gtccctctct | acttcccgcc | tgtctggagt | 18180 |
| ggctgtttct | attttttttt | aacactgctg | ttctggagag | tacacgcgct | ggagctgcct | 18240 |
| cccttagaag | ttctgtcgcc | gagctgctgt | agctgtgctg | gagcagcctg | ccaaagccgc | 18300 |
| agagctgtgt | gtgtcatctc | tacagcaatc | ctttacagcg | ccgcgcgctg | aagctgtgac | 18360 |
| aaaccccccg | gaactctctc | actctacagc | gctatctctc | cccc       |            | 18404 |

## 【圖 11】

[illegible]

## 【图 12】

[illegible]

【例 13】

|            |            |           |            |            |           |      |
|------------|------------|-----------|------------|------------|-----------|------|
| gxttoozgo  | tootoototg | tuttizgoz | gxttzgoz   | gxtztaot   | oogazgzz  | 4020 |
| ototggtta  | gxttoozot  | gxtgogog  | oogatzgzz  | gxtzttoog  | oogotgto  | 4080 |
| oogazggtt  | tgzgoogot  | ggxtgogoo | ggxtgogoo  | ggxtgogoo  | gagggto   | 4140 |
| gxtgoggtt  | gxtotgtta  | tattgtot  | oogotgtat  | gxttztgo   | gaggttgg  | 4200 |
| ttoagotgt  | oogotgtat  | oogotgtta | ttoototo   | gttgcattga | oattattgt | 4260 |
| oottgottta | ggatgattt  | oottgottt | gtttgtttg  | tgcaaaagc  | tgagagacc | 4320 |
| oogagattt  | gtttgtttg  | oogagattt | gxttgcattg | tattgtttg  | ggatgattt | 4380 |
| oogattggt  | ttotgttao  | oogagattt | oogagattt  | oogototo   | toogagaa  | 4440 |
| oogototot  | oogototot  | oogagattt | oogagattt  | ggatgattt  | ggatgattt | 4500 |
| gxttgggtt  | gxttgggtt  | oogagattt | ttotgagoo  | ggatgattt  | gaggttgg  | 4560 |
| gtttgtttt  | gtttgtttt  | gtttgtttt | gtttgtttt  | ggatgattt  | ggatgattt | 4620 |
| gtttgtttt  | oogotgttt  | oogagattt | oogagattt  | gtttgtttt  | ggatgattt | 4680 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | gtttgtttt | 4740 |
| gtttgtttt  | oogototo   | gtttgtttt | gtttgtttt  | gtttgtttt  | ggatgattt | 4800 |
| oogototo   | gtttgtttt  | oogototo  | gtttgtttt  | gtttgtttt  | ggatgattt | 4860 |
| oogototo   | gtttgtttt  | oogototo  | gtttgtttt  | gtttgtttt  | ggatgattt | 4920 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 4980 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5040 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5100 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5160 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5220 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5280 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5340 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5400 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5460 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5520 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5580 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5640 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5700 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5760 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5820 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5880 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 5940 |
| ggatgattt  | ggatgattt  | ggatgattt | oogototo   | gtttgtttt  | ggatgattt | 6000 |

## 【图 14】

|                  |                 |                 |                |                 |                 |      |
|------------------|-----------------|-----------------|----------------|-----------------|-----------------|------|
| ttgacagaca       | caagagocota     | acocotagoca     | cootacococa    | tgagacagoca     | ogagotagoca     | 6000 |
| tttgacagoca      | tgatotagoca     | ttatagagoca     | coococococa    | cagagagotoca    | ocagagococa     | 6020 |
| cootocotoca      | caagtagoca      | coacotagoca     | cootTTAGGT     | AHTTGGCATT      | GATGTAGTGC      | 6100 |
| GACCCGGGGA       | TTGTACTGTG      | CGGTGCGTGC      | AGGATAGTGC     | CGCTTGATGC      | ACAGtagoca      | 6180 |
| tgagagagaca      | gagagagatoca    | GTGTAGGTGT      | AGAGtagoca     | tacacacococa    | tgatagtagoca    | 6240 |
| octoctagoca      | tacacagococa    | ttatgttagoca    | agagagotoca    | agagotttagoca   | gagagatagoca    | 6300 |
| gagagagagaca     | tgagagtagoca    | ttatgttagoca    | gagagtagoca    | gagagtagoca     | agagtagtagoca   | 6360 |
| agagagagaca      | acagotagoca     | otagtagtagoca   | gagagtagoca    | gagagtagoca     | tagagagotoca    | 6420 |
| tttagagagaca     | agagagotoca     | gttagagtagoca   | tttagagtagoca  | gagagotagoca    | gagagtagtagoca  | 6480 |
| tacagagotoca     | tacagagtagoca   | gttagagtagoca   | gagagtagtagoca | gagagtagtagoca  | agagtagtagoca   | 6540 |
| gagagotagoca     | tgatocacagoca   | GGAGAAATGT      | CTTTATAGCG     | TTTCTGCCCT      | TTGTTCTGTG      | 6600 |
| CGCTTGCGCT       | TCCAGAGCGT      | GTGTCAAGAT      | ATTGACGCTG     | TGGTTCTAGCA     | ACGCTAGtagoca   | 6680 |
| tgagtagtagoca    | gagagtagtagoca  | tgagtagtagoca   | otagtagtagoca  | otagtagtagoca   | agagtagtagoca   | 6720 |
| gttagtagtagoca   | gagagtagtagoca  | gttagtagtagoca  | gttagtagtagoca | tgagtagtagoca   | gagagtagtagoca  | 6780 |
| gttagtagtagoca   | otagtagtagoca   | acagagtagtagoca | otagtagtagoca  | otagtagtagoca   | GAAGCTGTG       | 6840 |
| CGAGAGAGCA       | CGGTTGCGTG      | TATCTGTGCA      | CTCTGCTGTC     | TTGTTCAATT      | CGAGAGCTGC      | 6900 |
| GTGTTCAATG       | TACAGCGCAT      | TGACCGTGTG      | GGCATATATC     | GGtagtagtagoca  | agagagtagoca    | 6960 |
| agagtagtagoca    | acagagtagoca    | tacagagtagoca   | tagagtagtagoca | agagagtagoca    | agagagtagoca    | 7020 |
| GTAGAGGAGA       | tgagagtagoca    | acagagtagoca    | CGAGAGtagoca   | GAAGAGtagoca    | TGAGCGTGCTG     | 7080 |
| TTGATGCGTG       | TTTTTTTAAA      | ATTGTCAGCA      | AGGTGTGTGA     | GGCTGTGCGA      | AGTTGTGAAA      | 7140 |
| CGACCGAGTG       | TTGATGAGTG      | ACCGtagtagoca   | gagagtagtagoca | agagtagtagoca   | ocagtagtagoca   | 7200 |
| gttagtagtagoca   | ctagtagtagoca   | agottagtagoca   | tacagagtagoca  | gagagtagtagoca  | gagagtagtagoca  | 7260 |
| acagtagtagoca    | agagtagtagoca   | agagtagtagoca   | gagagtagtagoca | agagtagtagoca   | otagtagtagoca   | 7320 |
| GTGACAGTGA       | CTTTATATG       | AGAGtagtagoca   | CGAGAGtagoca   | CTGAGCGAGT      | GTGTGCTGTG      | 7380 |
| TTAGTGTAGA       | CGAGAGtagoca    | GAATGTGCTG      | CGGTTGTGTA     | GGCTGTGAGG      | CGAGAGtagoca    | 7440 |
| GTGAGAGGCT       | CGCGCTTTGC      | GTGACAGAGG      | GTGCTGTGCT     | GGCGCGGAGA      | CATGTGAGCA      | 7500 |
| TGTGTAGCAG       | tgagagtagoca    | agagtagtagoca   | gagagtagtagoca | ocagagtagtagoca | agagtagtagoca   | 7560 |
| ogagtagtagoca    | acagtagtagoca   | agagtagtagoca   | gagagtagtagoca | acagtagtagoca   | tgagtagtagoca   | 7620 |
| tgagtagtagoca    | gagtagtagoca    | ocagtagtagoca   | attagtagtagoca | otagtagtagoca   | tgagtagtagoca   | 7680 |
| tagtagtagtagoca  | attacagtagoca   | ttatagtagtagoca | gagagtagtagoca | agagtagtagoca   | attacagtagoca   | 7740 |
| gagtagtagtagoca  | ctagtagtagoca   | otagtagtagoca   | agagtagtagoca  | ctagtagtagoca   | acagtagtagoca   | 7800 |
| gttagtagtagoca   | gagtagtagtagoca | otagtagtagoca   | otagtagtagoca  | ocagtagtagoca   | gagtagtagtagoca | 7860 |
| otagtagtagtagoca | tacagtagtagoca  | ctagtagtagoca   | otagtagtagoca  | otagtagtagoca   | agagtagtagoca   | 7920 |



## 【 図 1 9 】

tmoocagat ttttttttt tagatgag gtatgtatat gtgocagag atgttttaa 15900  
 atootagag tagagatag ttoocotto gacotocaa atgtgtgaa toacagagt 15960  
 gogocooto atgtgttag gtittatott toacatoot agtotoaaa tigtatatac 16020  
 tigtgtgag atgtgttag toacatagag tootgtgaa oggtocoot gtotttttt 16080  
 ttttttttt caactagag atgtgttag toacatoot atgtgttag atgtgttag 16140  
 atgtgttag atgtgttag gacatoot atgtgttag atgtgttag atgtgttag 16200  
 gacatoot atgtgttag toacatoot atgtgttag atgtgttag atgtgttag 16260  
 gacatoot atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16320  
 gacatoot atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16380  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16440  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16500  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16560  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16620  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16680  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16740  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16800  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16860  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16920  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 16980  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17040  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17100  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17160  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17220  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17280  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17340  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17400  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17460  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17520  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17580  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17640  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17700  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17760  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17820

## 【 図 2 0 】

atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17880  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 17940  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18000  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18060  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18120  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18180  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18240  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18300  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18360  
 atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag atgtgttag 18404

## 【 図 2 1 】

ATGCTGTCCT GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 60  
 AAGGCGGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 120  
 TTCTGCTGT GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 180  
 GTATTGTATG ACGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 240  
 TCAGCAGAG ACGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 300  
 CCGCTGCTGT GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 360  
 CAGCAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 420  
 CTGCTGCTGT GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 480  
 GCTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 540  
 GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 600  
 ATGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 660  
 AATGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 720  
 ACAGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 780  
 TTGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 840  
 ATTCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 900  
 TCAGCAGAG TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 960  
 AAGCAGAG TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1020  
 GCTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1080  
 AACAAATGCT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1140  
 GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1200  
 CCGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1260  
 GACATGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1320  
 GCGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1380  
 GCGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1440  
 GAGTGTGAG TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1500  
 GTGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1560  
 AATAAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1620  
 ATGAGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1680  
 CAGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1740  
 AAGAGAGAG GTGCTGCTGT TCAGCAGAG CTACATGAG TGATGAGA GACCTGCTG 1794

## 【 図 2 3 】

MVRVFRDLGLDAETLLKGRGVHGSFLARPFRKNQGFSLVSRVGDQVTHIRIQNSG  
 DFYDLYGGEKFAITLVEYYTQQQGLQDRDGTIHLKYPLNCSPTSERWYHGHMSG  
 GQAEITLLQAKGEFWTLVRESLBPQGFVLSVSDQPKAGQSPRLRVTHIKVMCEGGRY  
 TVGGLETFLDSLTLVEHFKTGIEEASGAFVYLRQPYATRVNAADENRVLELNKKQDESE  
 DTAKAGFWEFEESLQKQEVKNLHQRLEGQRPNKGNRYNKLFFDHSRVLQGRDSNI  
 PGSDYINAIYKQLLGPDENAKTYIASQGLLEATVDFWQMAWQENSRVIMTTREVE  
 KGRNKCVPYWEVGMQRAYGPYSVTNCGEHDTEYKRLTLQVSPLDNGDLIREWHYQ  
 YLSWPDHGVPEPGGVLSFLDQINQRQESLPHAGPIVHCBSAGIGRTGIIVDMLENIST  
 KGLDCCDIDQKTIQVRAQRSGMVQTEAQYFYVIAQFIETTKKKLEVLQSKQDESEY  
 GNITYPAMKQAHAKASRTSSKHEDVYENLHTKNKREKVQKRSADKESKSLKRLK

## 【 図 2 2 】

| N   |     |                |            | C   |     |                |            |
|-----|-----|----------------|------------|-----|-----|----------------|------------|
| SH2 | SH2 | PITPase Domain | C-terminal | SH2 | SH2 | PITPase Domain | C-terminal |





## 【圖 30】

4020  
4060  
4140  
4200  
4260  
4320  
4380  
4440  
4500  
4560  
4620  
4680  
4740  
4800  
4880  
4920  
4980  
5040  
5100  
5160  
5220  
5280  
5340  
5400  
5480  
5520  
5580  
5640  
5700  
5760  
5820  
5880  
5940

[illegible]

## 【图 3 2】

[illegible]

|            |            |            |            |            |            |       |
|------------|------------|------------|------------|------------|------------|-------|
| tttggsgtt  | tttggsttgg | ggtttttttt | ggtttgggtt | gggttgggtt | gggttgggtt | 9990  |
| ggtttttttt | tttttttttt | tttttttttt | gttgggaaat | ttgtttttt  | tttttttttt | 10020 |
| ttttttttt  | ggaaattttt | tttttttttt | tttttttttt | tttttttttt | gggttttttt | 10080 |
| ttttttttt  | ttcgtttttt | ttttgggttt | ttgggttttt | gggttttttt | tttttttttt | 10100 |
| gggggggggt | tttttttttt | ggaaagtgtt | gggttttttt | tttttttttt | tttttttttt | 10140 |
| aaattttgtt | gatttttttt | tttttttttt | gttatttttt | ttgggttttt | tttggatttt | 10260 |
| ttttttttt  | tttttttttt | ttgggttttt | tttttttttt | tttttttttt | tttttttttt | 10320 |
| ttcgttttt  | tttttttttt | ggtttttttt | tttttttttt | tttttttttt | gttgggtttt | 10380 |
| ttcgttttt  | tttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | 10440 |
| ggattttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | 10500 |
| ttgggttttt | tttttttttt | ggattttgtt | ttggattttt | gggttttttt | ttgttttttt | 10560 |
| tttgggttt  | gggttttttt | gggttttttt | ttcgtttttt | gttttttttt | tttgggtttt | 10620 |
| gggttttttt | agtttttttt | ggattttttt | gggttttttt | tttttttttt | tttttttttt | 10680 |
| gggttttttt | tttttttttt | aaattttttt | tttttttttt | tttttttttt | agtttttttt | 10740 |
| tttttttttt | aaattttttt | gttgggtttt | gggttttttt | gggttttttt | gttttttttt | 10800 |
| aggttttttt | tttttttttt | tttttttttt | gttttttttt | tttgggtttt | tttttttttt | 10860 |
| tttttttttt | tttttttttt | gttttttttt | tttttttttt | tttttttttt | tttttttttt | 10920 |
| gggttttttt | aaattttttt | gttttttttt | gggttttttt | tttttttttt | tttttttttt | 10980 |
| agtttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | 11040 |
| tttttttttt | gttttttttt | tttttttttt | tttttttttt | gggttttttt | gttttttttt | 11100 |
| gggttttttt | tttttttttt | gttttttttt | tttttttttt | tttttttttt | tttttttttt | 11160 |
| tttttttttt | gttttttttt | gttttttttt | tttttttttt | gttttttttt | tttttttttt | 11220 |
| gttttttttt | tttttttttt | gggttttttt | tttttttttt | gttttttttt | agtttttttt | 11280 |
| agtttttttt | tttttttttt | gttttttttt | aaattttttt | tttttttttt | tttttttttt | 11340 |
| tttttttttt | gttttttttt | gttttttttt | tttttttttt | tttttttttt | tttttttttt | 11400 |
| tttttttttt | gttttttttt | gggttttttt | gttttttttt | tttttttttt | tttttttttt | 11460 |
| agtttttttt | tttttttttt | gttttttttt | gttttttttt | gttttttttt | tttttttttt | 11520 |
| aaattttttt | tttttttttt | gttttttttt | gttttttttt | gttttttttt | gttttttttt | 11580 |
| gttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | gttttttttt | 11640 |
| gttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | gttttttttt | 11700 |
| gttttttttt | tttttttttt | tttttttttt | tttttttttt | tttttttttt | gttttttttt | 11760 |
| aaattttttt | gttttttttt | tttttttttt | gttttttttt | gttttttttt | gttttttttt | 11820 |
| agtttttttt | tttttttttt | tttttttttt | gttttttttt | gttttttttt | gttttttttt | 11880 |

【图 3 3】

[illegible]

## 【图 3 4】

|            |             |            |            |            |             |       |
|------------|-------------|------------|------------|------------|-------------|-------|
| TTTTTGGTTG | GATATGCG    | gtgtgttttt | cgttttgttt | tattttggga | gttttttttt  | 13820 |
| gggttttttt | tttttttttt  | tggtgttttt | tggtgttttt | tggttttttt | ggagagagag  | 13880 |
| gggttttttt | ttttttgttt  | ttgtgttttt | GAGATTTGAT | TGGGAGAGAT | TGGTATTATT  | 14040 |
| AGGATTTGAG | TTGGTTGGAT  | TATGGGTTAT | TTAGTTGATT | TGGGGTGGAT | TTTATGTTAT  | 14100 |
| TGGATTAGAT | TAAATAGCG   | TGAGGAAGTT | TGTTTACGT  | AGGGTTTAT  | ATGGGTGAT   | 14160 |
| GTAGTGggg  | agctaatttt  | tggtgttgtt | agtgatgtt  | ggaggttaa  | tgttttgag   | 14220 |
| tttgttgtgt | tggttaagt   | attataaag  | tttgttgtta | tattgttgtt | tttgttgtta  | 14280 |
| tttgttgttt | tttttgtttt  | attataaag  | tttgttgttt | tggttgttgt | glttgttgtt  | 14340 |
| tttgttgttt | tttgttgttt  | attataaag  | tttttttttt | agatttgttt | tggttgttgt  | 14400 |
| tttgttgttt | atttgttttt  | attataaag  | agggatggg  | gatttgttgt | tttgttgttt  | 14460 |
| tttgttgtgt | tggttgttgt  | tggtgttgtt | tttgttgttt | gttgttgtt  | tggttgttgt  | 14520 |
| cgtttttttt | gggttgtttt  | tttgttgttt | tttttttttt | aggtatttgt | atgttgttgt  | 14580 |
| tttgttgttt | tttttttttt  | tttgttgtgt | tggttgttgt | gttgttgttt | tttgttgttt  | 14640 |
| tttgttgttt | tttgttgttt  | tttgttgtgt | tttgttgttt | gttgttgttt | gttgttgttt  | 14700 |
| tggttgttgt | tttgttgttt  | gttgttgtat | atttttgttt | tttttttgtt | cgatgttgtt  | 14760 |
| tggttgttgt | gttgttgttt  | gttgttgttt | gttgttgttt | tggttgttgt | tggttgttgt  | 14820 |
| atgttgttgt | tttgttgttt  | gttgttgttt | aggttgttgt | ggttgttgtt | gttgttgttgt | 14880 |
| tttgttgttt | tttgttgttt  | gttgttgttt | aatatttgtt | atttttgttt | tttgttgttt  | 14940 |
| tttgttgttt | aatatttgtt  | tttgttgtgt | gttgttgtgt | tttgttgtgt | tttgttgttt  | 15000 |
| gttgttgtgt | tttgttgtgt  | tttgttaaat | tggttgttgt | tttgttgttt | gttgttgttt  | 15060 |
| tttgttgtgt | aatatttgtgt | tggttgttgt | tttgttgtgt | gttgttgttt | atttgttgtt  | 15120 |
| tggttgttgt | gttgttgtgt  | gttgttgttt | gttgttgttt | tttgttgtgt | tttgttgttt  | 15180 |
| tggttgttgt | tggttgttgt  | gttgttgtgt | gttgttgtgt | tggttgttgt | gggttgttgt  | 15240 |
| tttgttgttt | tttgttgttt  | gggttgttgt | tatttgttgt | agttatttgt | tttgttgttt  | 15300 |
| gttgttgttt | tttgttgttt  | tttgttgttt | tttgttgtgt | tttgttgttt | aggttgttgt  | 15360 |
| aggttgttgt | gttgttgtgt  | tttgttgttt | tatttgttgt | tggttgttgt | atgttgttgt  | 15420 |
| tttgttgtgt | gttgttgtgt  | gttgttgttt | gggttgttgt | gggttgttgt | tttgttgttt  | 15480 |
| tttgttgtgt | tggttgttgt  | aggttgttgt | tttgttgttt | tttgttgttt | gttgttgttt  | 15540 |
| tttgttgtgt | tggttgttgt  | tttgttgttt | gttgttgttt | tttgttgtgt | aggttgttgt  | 15600 |
| gggttgttgt | aggttgttgt  | gttgttgtgt | cgatttgttt | tttgttgttt | gggttgttgt  | 15660 |
| gggttgttgt | gttgttgtgt  | atgttgttgt | atgttgttgt | gttgttgtgt | tttgttgttt  | 15720 |
| gggttgttgt | gggttgttgt  | tttgttgtgt | tggttgttgt | gggttgttgt | tggttgttgt  | 15780 |
| aggttgttgt | gttgttgtgt  | aggttgttgt | atgttgttgt | atgttgttgt | gggttgttgt  | 15840 |

【 ㊦ 3 5 】

[illegible]

## 【图 3 6】

|           |           |           |           |            |            |       |
|-----------|-----------|-----------|-----------|------------|------------|-------|
| atggaatg  | gagttgga  | ttagattha | gattttgga | tttttagatt | taacttttt  | 17680 |
| atttttagt | tattgttgg | gattttttt | gagagtttt | guttatttt  | tttttgatt  | 17690 |
| gatttttga | tgttgatna | gagagatgt | taattgttg | agagattat  | ttagattgt  | 18000 |
| ttagatttg | ttttttttg | ggagatgat | gataatttt | ttagatttt  | ggagttagtt | 18050 |
| tgttttttt | ttttttttg | tgattgttg | tittttttt | tttttttga  | attttgaag  | 18120 |
| tgttttga  | ggagagatt | ttgtttttt | gtttttttt | ttttttttt  | tgtttgaat  | 18180 |
| gagtttttt | ttttttttt | atatttttt | tttttttgg | taattgtgt  | ggagatttt  | 18240 |
| tttttttgg | tttttggtt | tttttttga | agtttttga | tattgtatt  | tttttagatt | 18300 |
| agatttttg | ggtattttt | tatttttat | tttttttat | tttttggtt  | attgtatttt | 18380 |
| atttttttt | ttttttttt | tttttttat | tttttttat | tttttttat  | tttttttat  | 18400 |



## 【圖 4 2】

7980  
8040  
8100  
8160  
8220  
8280  
8340  
8400  
8460  
8520  
8580  
8640  
8700  
8760  
8820  
8880  
8940  
9000  
9060  
9120  
9180  
9240  
9300  
9360  
9420  
9480  
9540  
9600  
9660  
9720  
9780  
9840  
9900

9060  
10020  
10080  
10140  
10200  
10260  
10320  
10380  
**10440**  
10500  
10560  
10620  
10680  
10740  
10800  
10860  
10920  
10980  
11040  
11100  
11160  
11220  
11280  
11340  
11400  
11460  
11520  
11580  
11640  
11700  
11760  
11820  
11880

【 図 4 4 】

11040  
12000  
12060  
12120  
12180  
12240  
12300  
12360  
12420  
12480  
12540  
12600  
12660  
12720  
12780  
12840  
12900  
12960  
13020  
13080  
13140  
13200  
13260  
13320  
13380  
13440  
13500  
13560  
13620  
13680  
13740  
13800  
13860

13920  
13980  
14040  
14100  
14160  
14220  
14280  
14340  
14400  
14460  
14520  
14580  
14640  
14700  
14760  
14820  
14880  
14940  
15000  
15060  
15120  
15180  
15240  
15300  
15360  
15420  
15480  
15540  
15600  
15660  
15720  
15780  
15840

## 【 図 4 5 】

tatttagat tttttttta tagatagag gttttttat gttttttag ttgttttaa 15900  
 atttttagt ttatgtatt tttttttta gttttttta agtttttag ttatagagt 15980  
 ggtttattt atttttagt gttttttat ttatatttt agtttttaa ttatttttt 16020  
 ttgtttggt ttgttttag ttatattag ttattttta agtttttaa ttatttttt 16060  
 ttgtttttt ttatttttag ttgtttttt attttttt ttgttttag agttttttt 16140  
 ttgtttatt agtttttag ttgtttttt tttttttt attttttt ttgtttttt 16200  
 gttttttt agtttttag ttgtttttt ttattttt ttattttt ttattttt 16260  
 gttttttt agtttttag ttgtttttt tttttttt tttttttt ttattttt 16320  
 gttttttt agtttttag ttgtttttt ttattttt ttattttt ttattttt 16380  
 attttttt ttattttt ttattttt ttattttt ttattttt ttattttt 16440  
 ttgtttttt ttattttt ttattttt ttattttt ttattttt ttattttt 16500  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16560  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16620  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16680  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16740  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16800  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16860  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16920  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 16980  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17040  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17100  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17160  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17220  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17280  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17340  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17400  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17460  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17520  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17580  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17640  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17700  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17760  
 ttattttt ttattttt ttattttt ttattttt ttattttt ttattttt 17820

## 【 図 4 6 】

agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 17880  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 17940  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18000  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18060  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18120  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18180  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18240  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18300  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18360  
 agtttttag ttattttt agtttttag ttattttt agtttttag ttattttt 18404

## 【 図 4 7 】

(a)

Wild type DNA

5'-AGCTC8CGATGCCAGCTGCTCG-3' sense strand  
 3'-TCAGCGCTACGTTGAGCGAGC-5' antisense strand

(b)

Blauflied

5'-AGTTCC8CATGTTAGTTGCTTCG-3' sense strand  
 3'-TTGAGCGCTATGTTGAGCGAGC-5' antisense strand

(c)

FW primer

5'-AGTTCC8CA  
 5'-AGTTCC8CATGTTAGTTGCTTCG-3' sense strand  
 3'-TCAAGCAAGC-5' RV primer

(d)

FW primer

5'-AACTC8CGA  
 3'-TTGAGCGCTATGTTGAGCGAGC-5' antisense strand  
 3'-TTGAGCGAGC-5' RV primer

## 【 図 4 8 】

(a)

REP-S1 : 5'-CAGGCCAGTGGAGTGGCAG-3'

(b)

REP-AS1 : 5'-GAGGAGGTGACGCTAGTCTG-3'

(c)

(H7441)

CAGGCCAGTGGAGTGGCAGGCCAGAACTGGGACCAACCGGGGCTGCTGA

REP-S1

HpaII

GGCGGCGCGGCACTGGAGCTGCATCTGAGGCTTAGTCCCTGAGCTCTCT

HpaII

GCCTGCCAGACTAGCTGCACCTCTC (H7596)

REP-AS1

## 【 4 9 】

(a)

REP-S2 : 5'-GAAAGCACTGGCTTTGGAAC-3'

(b)

REP-AS2 : 5'-ATCGAGTGAATCCTGCTGAT-3'

(c)

(#8858)

CAAAGCACTGGCTTTGGAACCGGACTGTCTGGTTTGAATCCTGGCACTG

REP-S2 Hpa II

CAGCTGACTCACTGATGAGTCAAGCAATGCCCTAAACTCCCTGAGCCTG

AGTTTCCTTGTCTGTAAATGATAAGATAGCCCTGTTTCATAGGGCTGT

GGTGAGAAACCAATCAGACAAGGCATGTGAACGCCATTATAGCAGAGG

CCCGCATCCAGCAGGACTCACTGAT (#7084)

Hpa II REP-AS2

## 【 5 0 】

(a)

SHP1-PF1: 5'-TGTCTGGAGGCCACGGTCAATGA-3'

(b)

SHP1-PR1: 5'-GTTTGTATTGGTTGTGTGTCATGCTC-3'

## 【 5 1 】

(a)

SHP-LF1: 5'-CCGAGTTCATTGAAACCACT-3'

(b)

SHP-LR1: 5'-CGTTGCTTGTCTGCTTGTGT-3'

## 【 5 2 】

(a)

MF2 : 5'-GAACGTTATTATAGTATAGCGTTC-3'

(b)

MR2 : 5'-TCAGGCATACGAACCAACG-3'

(c)

(#7037)

GAACGCCATTATAGCAGCGCCCGCATCCAGCAGGACTCACTGAT

MF2

GACAGTTGTACCGCCATCATTGTTATTAGCGTGGGCCAGGAGGCT

GCGTAAAGCAGCTGCTGGAGGAGGAGATGCCGTGGGACCGTCT

GGGTTCCGATCGGTGA (#7105)

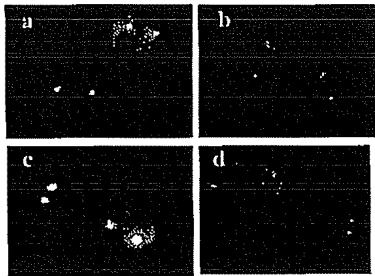
MR2

## 【 5 3 】

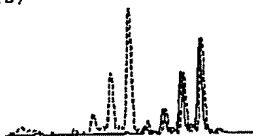
(a)

FISH analysis of ILTMot and NK-YS cells with chromosome 12 or SHP1-specific probes

| cells  | probe  | positive signal No.(%) |   |    |   |   |      |
|--------|--------|------------------------|---|----|---|---|------|
|        |        | 0                      | 1 | 2  | 3 | 4 | more |
| ILTMot | Ch #12 | 1                      | 1 | 97 | 1 | 0 | 0    |
|        | SHP1   | 1                      | 2 | 95 | 1 | 1 | 0    |
| NK-YS  | Ch #12 | 0                      | 0 | 99 | 1 | 0 | 0    |
|        | SHP1   | 1                      | 3 | 91 | 4 | 1 | 0    |



(b)



| Microsatellite marker | LOH          |
|-----------------------|--------------|
| D12S356               | 15/19 (79 %) |
| D12S336               | 6/16 (38 %)  |

## フロントページの続き

| (51) Int. Cl. <sup>7</sup> | F I   | テーマコード (参考) |
|----------------------------|---|-------------|
| G 0 1 N 27/447             | G 0 1 N 33/53   | M           |
| G 0 1 N 33/53              | G 0 1 N 33/566  |             |
| G 0 1 N 33/566             | C 1 2 N 15/00   | A           |
|                            | G 0 1 N 27/26   | 3 0 1 A     |
|                            | G 0 1 N 27/26   | 3 1 5 J     |
| F ターム (参考)                 | 4B063 QA01 QA13 QQ08 QQ33 QQ43 QQ53 QQ62 QR08 QR14 QR32 |             |
|                            | QR50 QR62 QS11 QS16 QS25 QS34                           |             |